

Opportunity ahead for Europe

The European Commission has enormously improved its administration of the Community's research programme. But there is a long way to go.

THE European Community's research programme continues to become more tangible and hard-headed. Several months have already passed since, in an almost once and for all break with precedent, the European Commission provided a meeting of research ministers from member states with an agenda that was well prepared (see *Nature* 297, 613 and 619; 1982). The preparations for next month's meeting in the same series are even better — there is less paper but more time in which to read it (see page 188). The chances are therefore good that the participants in next month's meeting will retain a clear sense of what they have accomplished, and will thus be doubly well prepared to take part in the next meeting. The way things are going, it is no longer outrageously overhopeful to suppose that meetings of what is called in Brussels the Science Council will be memorable for the constructiveness of their discussion and the clarity of their decisions. Yet it is too soon to know when the European Community will have a policy for research and development that is at once appropriate and effective.

The difficulty is constitutional, and not of the Commission's making. The Commission is not a government but, rather, an organization whose existence is assured by the Treaty of Rome but whose role is only partly defined by what the treaty says. The Commission is required to stamp out restraints on competition between member states, if necessary by taking member governments to the European Court or by promulgating directives which have the force of law. It also has explicit treaty obligations for the administration of European agricultural policy. But in other fields, such power as the Commission enjoys depends on the willingness of member governments to relinquish responsibility to Brussels. It is not in the circumstances surprising that the hard core of what the Commission is pleased to call its "research, development and demonstration" programme should consist of projects such as in thermonuclear fusion; none of the member governments would think it worthwhile supporting a viable programme of research of its own, but most of them fall in with the proposition that something substantial should be done collectively. The result is that the Commission is now proposing to spend 480 million European Units of Account (the paper currency unit now almost exactly equal to one US dollar once again) on fusion over the next three years (most of it on the Joint European Torus, much of the rest on the Next European Torus). This also explains the Commission's substantial research programme on safety aspects of fission reactors. Member governments find the arrangement convenient; otherwise, they would not foot the bill.

But need the planning of the Community's research programme always have this makeshift character? While the Treaty of Rome remains the Community's rule-book, and for as long as the day to day conduct of the Community's affairs is determined by short-term calculations of narrow self-interest, there is probably no alternative. If the Commission is ever to win member governments' consent to a less grudging central research programme, the following conditions will have to be satisfied.

- Central expenditure should threaten nobody but be a convenience to member governments. This condition is satisfied by the collaborative research programme on thermonuclear fusion but was not satisfied by the Community's ambitious plans more than a decade ago to launch a collaborative research programme on computer design (which is why nothing ever came

of it). The problems with which the Community must learn to live are entirely familiar to those who manage research programmes whose cost is met by contributions from members which are ordinarily competitors of each other. So substantial opportunities for spending money centrally will be found in large long-term projects (fusion), public service projects (nuclear waste disposal), basic research (in most fields), and those which are best dealt with collaboratively (such as the effects of acid rain).

- Centrally administered research must not create a huge establishment of its own. The Euratom research organization failed primarily because its plans for reactor-types were too fanciful, but its one-time existence is still marked by the survival of the Community's Joint Research Centre at Ispra. Making fuller use of this and establishments that cannot simply be closed down remains a continuing practical preoccupation for Brussels even though it should in due course wither away.

- The Community's selection of projects to support should carry the same kind of conviction as do the programmes of the more reputable grant-making agencies. One obvious snag is that conventional techniques of peer review are not easily applicable across the whole of such a wide field, and in circumstances in which over-pious statements of general objectives are likely for a long time to be the rule. Qualitatively, however, the Commission's practice in the past few years seems to have been heading in the right direction.

Quantitatively, there is a long way to go. The agenda paper for next month's Science Council points out that the total spending on research and development within member states amounts to a fifth of the world's total, and that European member governments collectively spend on research and development 70 per cent of what the United States spends on these activities and more than three times what the government of Japan affords — a slightly confusing comparison because the ratio of private to public research in Japan is more like 70:30 than 50:50 as in Europe and the United States. The implication is that Europe collectively could give its principal industrial competitors a run for their money on research and development. Mercifully, next month's agenda paper stops short of implying that all this would happen if only governments would hand over to Brussels a larger proportion of what they spend on research and development. Realism seems to have supervened.

So in what directions should the new research team at Brussels be hoping to expand? The most obvious opportunity is in basic research. The Commission is exceptionally well placed, being qualified to spend funds on multinational projects when member states might be prevented from doing so by domestic legislation. The European Science Foundation has shown that there are plenty of opportunities for using modest sums of money sensibly, especially on assisting collaboration between groups of people or between institutions. The Commission should be aiming to take a leaf out of the foundation's book — the best immediate opportunity for spending money in ways that will quickly improve the quality of European research. Further ahead, however, the objective must be to monitor the pattern of European trade in high technology to find ways in which the changing division of labour between the members of the European Community makes sense of centrally sponsored research. This will be a slow job, but it is both the best and the only way forward.



No law in the jungle

Mr Eugene Rostow's departure from the Reagan Administration whatever the reasons, is a setback.

DURING President Johnson's time, Mr Eugene Rostow was widely considered to be something of a hawk, a common fate of conservative Democrats in the 1960s. It is therefore ironical that Mr Rostow should now have been forced out of his post as director of the Arms Control and Disarmament Agency after several months during which he has been more frequently been accused of being too dovish (see page 189, this issue). Even though the *New York Times* was last week telling its readers that Mr Rostow's departure had been precipitated by his willingness to modify President Reagan's proposal that the intermediate nuclear weapons negotiations at Geneva should aim at the "zero option", it has been clear for months that Mr Rostow's authority would be steadily undermined by the unwillingness of the US Senate to let him appoint his chosen deputy, a struggle in which neither the White House nor the State Department seems to have shown much interest.

What happens next is anybody's guess, but what has happened in the past few days should suggest ways in which the United States (whatever the colour of the administration) could more effectively conduct negotiations on arms control. One of the perennial problems of Washington is that responsibility does not lie where it belongs. The secretaries in charge of the most powerful departments of state frequently find themselves outgunned and even undermined by the small group of close advisers by whom the president for the time being is surrounded. How much more difficult must it be for somebody in Mr Rostow's old shoes? For while the arms control agency is titularly a free-living organism, in the sense that the Congress looks into its affairs as if it were autonomous, the enabling legislation requires the agency often to behave as if it were part of the State Department. The result is that either the agency functions forthrightly and independently, thus making the cause of arms control prosper but running the risk of giving offence to the State Department, or it gives no offence but gets little done. Either way, the result is unsatisfactory.

This is why it has been clear that, whatever the value of the fiction that the agency is autonomous during other administrations there is no reason to think that it is compatible with President Reagan's way of running things. Indeed, in the wake of Mr Rostow's departure, and because of Washington's new-found understanding of the importance of meeting public opinion on arms control, three contradictory signals have been put out from Washington — a successor to Mr Rostow has been appointed, suggesting business as usual; it is being said that the Secretary of State, Mr George Shultz, will take a closer interest in arms control; and it is also being said that President Reagan will make the subject his personal concern. On the face of things, this is a recipe for the repetition of disaster.

The moral is plainly what it has been for several months — the arms control agency should be made unambiguously part of the State Department, with responsibility for its health and welfare squarely on the shoulders of the Secretary of State. The belief that the cause of arms control could only be magnified by the existence of a separate agency with responsibility for arms control is denied by the long delay, at the beginning of the Reagan Administration, before Mr Rostow's appointment and the public demonstration since that even a powerful holder of that office can be thrown to the wolves if the price of keeping him is too great. The separate existence of the Arms Control and Disarmament Agency is as much a hazard for the cause of arms control as it is a way of institutionalizing the cause.

In the jungle fighting of the past few days, the most serious casualty is likely to have been not Mr Rostow but the prospect that the Reagan Administration, slowly waking to the need to respond to what Mr Andropov has been saying about arms control, might have been persuaded by the momentum of events to make a constructive or even an imaginative response. Now, it is more likely to be pleading for time in which to consider all the newly

presented options, also to consider what Mr George Bush will learn on the European journey not yet begun and, ideally, also to win some kind of resolution of domestic questions such as where to put the MX missiles (and how to pay for whatever solution seems best in 1983). By then, unfortunately, a great deal will have happened in Europe. The general election in West Germany is, for example, barely six weeks away.

Seeing morning stars

The BBC should abandon its plan to peddle astrology. What people need is reality.

AT A TIME when the British Broadcasting Corporation (BBC), thinking itself threatened by cable television, refers to the merits of the principle of "public service broadcasting" with every breath, it has astonished even its friends by hiring an astrologer as part of its breakfast-television team. This service, begun this week, anticipates by two weeks a commercially supported breakfast show. The corporation's explanation of its venture into astrology, in no way a defence, is that there are many people who will not start the day without knowing what the stars hold for them. This is yet another snook cocked at the memory of Lord Reith, the corporation's founder and first director-general, whose posthumous influence until recently kept the corporation's broadcast output innocent of news of horse-race betting (but now there is a tipster on Radio 4). What would he have made of the peddling of horoscopes every morning?

The corporation claims that its man, once dubbed "astrologer royal" on the grounds that his advice had been sought by a member of the British royal family, is the "best in the business". By what measure, pray tell? How is it possible to judge the success



of predictions which, even when by accident correct, are based on falsehood? How does Reith's institution plan to grapple with the unpalatable truth that the stars do not influence behaviour. People born at the same minute of the same day do not share a common fate. People killed by the same bolt of lightning are not born on the same day. Those who strike up a friendship because they happen both to be Sagittarians must be supposed to be deficient in the ways of courtship or conversation. And those who think otherwise are a pernicious influence in society. So what price, now, public broadcasting?

The truth is, of course, that the competition for audiences takes precedence over principle. Elsewhere, it must already seem anomalous that the established public broadcasting service should put out what would be unthinkable in a country in which church and state were separated — the *BBC Hymn Book*. Is it just possible that the corporation, now aware that Britain is less homogeneous than it used to be, equates astrology with the Christian and other religions that now occupy its frequent "God slots"? The identity is false. Astrology is sheer superstition, all the more pernicious because so many people think otherwise. Broadcasting astrology over monopoly-run air waves, without explaining that people are being hoodwinked, is a public disservice, however successfully it may win an audience for breakfast television. But to that end, astrology is probably only the thin end of the wedge. Why not advice on cooking for the Vegan diet, on how to talk to plants or communicate with the dead?

US cancer research

NCI "mismanagement" causes unrest at Frederick

Washington

THE neat signs used to say "Frederick Cancer Research Center" on the Maryland campus redeployed by President Nixon in 1971 from US Army biological warfare research to the war on cancer. But now, since October 1981, the signs say "Frederick Cancer Research Facility", and the campus is far from happy.

The change of name is symbolic to many who work in the 67 low drab-looking buildings on 70 acres of US Army land at Fort Detrick. Many talk earnestly about Frederick's quest to become the "centre of excellence" that Nixon, at the dedication ceremony, said it should be. They say they do not want to work at a "facility" and that the change is symptomatic of how the National Cancer Institute (NCI) has over-managed them and forced some to go job-hunting.

A recent string of management decisions by NCI involving Litton Bionetics Inc, the chief contractor, Litton's strong-willed manager, Michael G. Hanna Jr, Johns Hopkins University and four other companies which now run the place with Litton, have some of the staff up in arms.

"The place is a great recruitment ground now", says Hanna, whose abrupt departure after building it up from a \$2.5 million per year centre in 1975 to approximately \$30 million last year, has been one cause of concern.

James L. Liverman, a former Department of Energy official brought in by Litton to run the basic research until a permanent leader is chosen, agrees that things have been going badly since the new regime began on 27 September, with five contractors, instead of just Litton, running the "facility". To help, Liverman has set up "transition groups" where different sides meet and talk out their problems.

But one scientist, who is rumoured to be job hunting, sees the year of problems and surprises as jeopardizing the goal of "a centre of excellence". The centre's reputation grew, in part, because good laboratories were induced to move the 35 miles from NCI's campus in Bethesda.

Peter J. Fischinger, associate director of NCI with responsibility for Frederick, says each of the recent decisions was made so that the government could be sure it was getting its money's worth at Frederick. He explained that since its opening in 1972, Frederick has been the object of suspicion by NCI grantees, congressmen and budget officials, because it is a contract programme, because it has grown like Topsy when other budgets have levelled, and

because some of the science may not be first-rate.

Others agree that the real issue has been NCI's attempt to control Frederick more closely in response to these criticisms.

Litton has held the contract to manage Frederick since 1972 and was the only bidder when a second five-year contract came up for competition in 1977. In response to complaints of unfairness, NCI let it be known in advance of the second competition in 1982 that the management contract would be split into five.

Many on the Frederick campus were intrigued by the fact that Litton's main competitor for the \$8 million basic research programme was Johns Hopkins University. In August it was announced that the £30 million contract for operations and technical support would go to PRI — a small \$5 million-a-year Rockville company. In late August, Harlan Sprague Dawley won the \$1.6 million contract to run the animal breeding service. In early September it was announced that Litton, not Johns Hopkins, would run the basic research programme. A small company was chosen for the computer service in September and the library contract was announced on 24 September, the Friday

before the Monday when all five would start running Frederick together.

Many even at NCI are puzzled that Hopkins did not win. Mark Pearson, the geneticist who was Hopkins' candidate to run the contract, says that the possibility of having graduate students and members of Hopkins' faculty linked with Frederick seemed likely to move Frederick light years closer to being a true centre of excellence.

An NCI official said Hopkins had lost because its bid would be more costly than Litton's and that the proposal entailed only the biology department interacting with Frederick. A Hopkins source, however, estimates that Hopkins' bid could have been only \$200,000 more than Litton's, and Hopkins had proposed a broad science-management advisory committee to assure complete Hopkins participation.

The real reason behind Hopkin's defeat is said to be that the evaluators asked whether Hopkins would be willing to undertake research requested by NCI outside the scope of the contract. Hopkins said maybe not.

A final cause of anxiety is the reprogramming of up to \$125 million in funds in the contract, now being undertaken at the recommendation of yet another group, a scientific review of the entire Litton basic research programme there, headed by Paul Zanecnik. NCI says the reprogrammed funds may be used by the new principal investigator, perhaps to concentrate on oncogene research. But many Litton scientists (who work alongside NCI scientists at Frederick) are wondering if Litton will be out in another five years.

Deborah Shapley

US nuclear power

Split vote on NRC safety limits

Washington

WAVING aside objections from its technical advisory group, the Nuclear Regulatory Commission (NRC) last week adopted a new safety standard for nuclear power plants that would for the first time define what level of safety is adequate. The standard, not legally binding during a two-year trial period, sets a goal of one core-melt accident per 10,000 reactor-years.

The standard also calls for the risk of accidental death from nuclear power plant operation not to exceed 0.1 per cent of the average background risk experienced by the general population, both for immediate death and cancer death. Those background risks are 5 in 10,000 per year for accidental death and 20 in 10,000 per year for cancer death, according to NRC staff member Robert Bernero.

Bernero said that of 16 plants studied, 7 exceed the safety goal "in one respect or another". He said that the core-melt risk varied from a factor of 10 below the goal to a factor of 10 above in the plants examined.

NRC's Advisory Committee on Reactor Safeguards, in a letter to the commission,

objected to several aspects of the new standard. While not opposing the explicit safety goals, the advisory committee wanted to retain the present standard of reducing risk to a level "as low as reasonably achievable". The advisory committee also advocated integrating cancer risks over the entire surrounding population of a plant, rather than limiting the calculation to a 50-mile radius.

The new standards will not act as regulations in themselves, but will be used by NRC as guidelines in deciding what safety equipment and modifications to require in power plants. Bernero said that, nonetheless, "the mere existence of the standards would press for improvements" in plants that exceed the goals. But NRC member Victor Gilinsky — the sole opponent of the new standard in the commission's 4-1 vote — said the effect would be to "place a cap on regulation, not on risk". Gilinsky was unsuccessful in his attempts to persuade the other commissioners even to meet the advisory committee to discuss their objections.

Stephen Budiansky

Seeking extraterrestrial life

NASA is not quite alone

Boston

HAVING weathered a Golden Fleece award and, last year, a cut-off of funds, the National Aeronautics and Space Administration (NASA)'s Search for Extraterrestrial Intelligence (SETI) programme is back on its feet. This spring, the centre-piece of the programme — a multichannel analyser to scan 74,000 radio channels simultaneously for evidence of non-natural signals — will go on-line. And, confident that funding will continue, NASA has a five-year research and development plan that will eventually expand that capability to eight million channels.

The programme's recent financial troubles had seemed likely to kill SETI once and for all. Senator William Proxmire (who, in 1977, had awarded SETI one of his Golden Fleece prizes for projects that represent the biggest wastes of taxpayers' money) succeeded in attaching an amendment to NASA's appropriation for fiscal year 1982 forbidding the agency to spend any of its funds on SETI. NASA spent a year shutting the programme down, only to have it revived this year after a visit from Carl Sagan apparently persuaded the senator to mute his objections. NASA will spend \$1.5 million on SETI this year.

Criticism from within the astronomical community, however, persists. The latest row was set off by a letter by Dr Frank Tipler of Tulane University that appeared in *Science* last week. Tipler takes Carl Sagan to task for having argued that the SETI radio search will have "profound implications for our view of the Universe and ourselves", whether the results are positive or negative. Tipler says that "a negative result would probably not convince supporters of SETI that extraterrestrial civilizations do not exist. They could simply argue that advanced civilizations generally abandon inefficient radio transmitters like ours after a short time . . . SETI will only become a science when its proponents tell us what observations will convince them that it is reasonable to assume we are alone".

Tipler's criticisms did not impress SETI proponents attending the American Astronomical Society meeting in Boston last week. Dr Jill Tarter of the University of California, Berkeley said, "Tipler's arguments never did have validity"; Dr Frank Drake of Cornell University called them "a lot of baloney". In fact, Drake said, "there's no way ever to prove the negative" in any scientific experiment.

SETI supporters also pointed to the National Academy of Sciences study last spring on priorities in astronomical research (the Field committee report) which endorsed a modest SETI effort and to the establishment of a committee on extraterrestrial life by the generally staid International Astronomical Union (IAU)

as evidence that they have the astronomical community behind them. Drake said that he has been "deluged with requests" from IAU members seeking to join the committee since its establishment last August.

Nonetheless, the underlying question in Tipler's recent criticism may have hit a raw nerve. SETI proponents seem painfully aware of the limitations inherent in the current research plan. Even with the full eight-million-channel real-time capacity, the analyser will cover a spectrum of only 8 MHz at one time. Each channel is 1 Hz wide in order to screen out natural astronomical phenomena, which have bandwidths of at least 1 kHz.

But even the constraints imposed by natural noise (which interferes with signals at wavelengths longer than 10 m or so) and by thermal radiation (which interferes at



wavelengths shorter than 5 cm) leave a useful listening band 1,000 times broader than the 8 MHz capability of the analyser.

Similarly, limiting candidate stars to those nearby and those similar to our Sun leaves tens of thousands to be studied.

Thus it will always be easy to argue that the lack of a positive result is due to having looked in the wrong place — and thus easy to continue on what may in fact be a wild-goose chase. Tarter maintained that "it would be hard in a single lifetime to amass a statistically significant negative result".

Another difficulty that SETI may face is some foot-dragging, even on the part of enthusiasts — particularly enthusiasts who happen to be observatory directors. As Dr Stuart Bowley of University of California, Berkeley — a SETI researcher — said, "the intrinsic problem is that you must take over a radio telescope to conduct the search", taking away limited observation time for a project that, as Bowley readily admits, has an exceedingly small chance of success.

And for all the claims that SETI has achieved respectability within the astronomical community, the general scientific community remains sceptical. The National Science Foundation has no official policy on SETI research, but refers enquiries to the NASA programme.

Stephen Budiansky

NASA astrophysics budget

Hope springs eternal

Boston

DESPITE a flurry of last-minute budget revisions ordered by President Reagan to bring down a federal deficit projected to exceed \$200,000 million, officials of the National Aeronautics and Space Administration (NASA) and other members of the astronomy community remain confident that they will fare well under the 1984 budget, due to be released on 31 January.

"I still believe that they will stand up and support the good, solid projects", Frank Martin, director of NASA's astrophysics division, said here at the American Astronomical Society meeting. Martin suggested that much of the credit for the favourable reception that astronomy is receiving at the White House belongs to the Field committee report, issued last spring, which ranked by priority various astronomical projects (see *Nature* 296, 482; 1982). It is widely viewed as reflecting the consensus of the astronomy community, and represents the sort of priority-ranking that White House science adviser George Keyworth has been urging all disciplines to establish. "If you can get the community to agree on what to do, it's a very powerful statement", Martin said.

NASA will reportedly get the go-ahead for two major projects in the 1984 budget: the Very-Long Baseline (VLB) array and the Venus radar mapper. The VLB array, to consist of 10 radio telescopes, would permit an angular resolution of 0.3 milli-seconds of arc, a hundred-fold improvement over current observational limits. The Field committee had recommended spending \$50 million on the project over the decade.

The Venus radar mapper is the scaled-down version of the VOIR (Venus Orbiting Imaging Radar), born of NASA's realization that the only hope for approval of the mission was in cutting its \$600 million cost. The Venus radar mapper will cost exactly one-half of that; it will also provide a 1-km resolution of Venus's surface, ten times degraded from the original specifications. In almost any form, though, approval of the mission would provide a much needed shot in the arm for the planetary exploration programme, whose last new project was the Galileo mission to Halley's comet.

NASA is meanwhile proceeding swiftly on the space telescope and the gamma-ray observatory and on plans for the project ranked first by the Field committee, the advanced X-Ray Astrophysical Facility (AXAF). AXAF will provide a sensitivity 100 times greater than that of the Einstein observatory (which "died" in orbit in 1981) and an on-axis resolution eight times greater. It will also fill a 10-year gap (by the

time of its launch in 1990–91) during which the United States will have had no orbiting facility at all for X-ray data.

NASA is planning to issue next month a request for proposals for the detailed design ("phase B" in NASA's lingo) of AXAF. Proposals for focal-plane instruments will be due in July. NASA is hoping to receive approval to begin construction in the 1986 budget.

Dr George Clark of Massachusetts Institute of Technology noted that before the Field committee report, the launch date for AXAF had "receded at a rate of one year per year". Now, he said, "AXAF seems firmly set in NASA's planning".

NASA also hopes to receive approval in the 1984 budget for an expansion of the Explorer satellite programme, which the Field committee ranked as top priority among "modest" new programmes. The new Explorers would include satellites for studying the extreme ultraviolet, X-ray timing and the solar corona.

Martin also expressed confidence that the new projects would not come at the expense of other parts of the new programme. He admitted that data analysis "suffered from the 1983 budget-crunch". But "without the flight projects", he said "the whole programme atrophies".

Stephen Budiansky

Blood-typing reagents

Monoclonal product on UK market

CELLTECH Ltd, the specialist British biotechnology company, has started bulk manufacture and sales of monoclonal antibody ABO blood grouping reagents made by artificially-produced hybrid cells. This is the first bulk-scale application of mammalian cell hybridization. The reagents will be cheaper than commercial products made by conventional methods, and are claimed to be more reliable.

Early development of the new reagents was carried out by a team under Dr Ed Lennox at the Medical Research Council's Laboratory of Molecular Biology at Cambridge. The Medical Research Council, which by standing agreement offers its monoclonal antibodies to Celltech for manufacture, then licensed Celltech to proceed to bulk production.

The story, like some other notable discoveries, has a fairy-tale beginning: an antibody with anti-A specificity was found unexpectedly during an experiment designed to look for tumour antibodies. The accidental product was found to be a more effective reagent for blood typing than those now in use in Britain, which are mostly extracted from blood given by selected donors. However, its properties fell short of those of other commercially produced reagents obtained from blood given by hyperimmunized volunteers. The work of the Cambridge team was then to find mouse lines and immunization schedules suitable for the production of anti-A antibodies with high avidity and correct specificity. The other reagent, anti-B, was also soon being produced, and is now claimed to be better than any other available.

Having perfected the product, the scale-up operation to bulk production — complicated by the physical fragility of the cells — was handed to Celltech, which substantially increased yields. By using automated monitoring equipment, Celltech was able to identify optimal physicochemical conditions for bulk production of the cells while maintaining high rates of antibody production. The cells are now grown in 100-litre vessels, and

a continuous-production system is being developed.

Because each is a single antibody, the new reagents have substantial advantages over products made by conventional means, which contain a variable mixture of different antibodies. Their use will also conserve supplies of blood, which is not used in the production process.

Celltech's products came through with flying colours in a large-scale study involving more than 85,000 tests co-ordinated by the National Blood Group Reference Laboratory: Celltech claims that the new reagents produce no "false positives". The tests employed were based on the exacting US Food and Drug Administration tests, and Celltech is confident that its new products will be approved and be competitive in the American market, as well as the European and Japanese markets. Deliveries to several UK blood transfusion centres have already started. According to Dr Tim Leeney, the Celltech business manager leading the project team, monoclonal antibodies will take a significant share of the world market for blood grouping reagents by the end of this decade. The Department of Industry, which recognizes a good thing once it has been pointed out, has awarded Celltech a grant for the study of continuous cell culture.

In the course of the coming year, blood-typing reagents will be supplied within the National Health Service free of charge, with financial support thought to be of the order of £100,000 from the Department of Industry. It is hoped that during the year in question, overseas markets will be opened up and that it will also be possible during that period for the Department of Health to make a realistic appraisal of the true cost of supplying conventional typing reagents.

The expertise Celltech has acquired in the large-scale use of hybridomas puts it ahead in the rush to develop other monoclonal blood grouping reagents, including rhesus-specific agents. Celltech considers that further reagents of this kind will be available within two years. **Tim Beardsley**

Acid rain research

New panel's industrial bias

Washington

THE Reagan Administration's commitment to acid rain research, already under question, has come under a new cloud. Last month when the president named to the panel overseeing federal acid rain research three new members, all three turned out to have close ties to the utility industry.

Earlier last month, the Administration provoked protests from environmental groups and several members of Congress when the Office of Management and Budget (OMB) directed the Environmental Protection Agency (EPA) to cut funding for the development of an acid rain model from \$650,000 to \$150,000. The model, to be used in assessing the effects of utility emissions control strategies on sulphur and nitrogen emission, was to have been ready for use next year. According to EPA, it will now be completed in 1986, by which time a similar model for industrial sources will also be completed.

The OMB action was considered unusual in that it effects funds already appropriated and being spent — and that it deals with a very detailed internal policy decision. OMB could not actually order a cut; what it did was to direct EPA to "reprogramme" \$500,000 from the utility model to the industrial model. But according to the Natural Resources Defense Council, EPA sources say that at most \$200,000 of the reprogrammed funds could actually be used this year on the industrial model.

Environmental groups had been eager for EPA to complete work on the utility model, which will be available for public use. Groups such as NRDC have been spending large sums on hiring consultants to run model predictions for policy analyses.

The appointments of the three new members to the Acid Precipitation Task Force has added fuel to the fire. The task force, which is made up of representatives of the 13 federal agencies involved in the 10-year research effort plus four outside members, oversees all aspects of the Federal Government's research programme on acid rain.

The new task force members, who are not subject to Senate confirmation are: Ralph Perhac, director of environmental assessment at the Electric Power Research Institute (the electric utilities' research organization); James Mahoney, vice-president of Environmental Research and Technology, a consulting firm that has many contracts with industry and the government, and Professor John McKetta, of the University of Texas at Austin, who has frequently served as an expert witness for the utility industry.

Stephen Budiansky

European research policy

Brussels to establish order?

Brussels

THE grand strategy for reorganizing and expanding the European Communities' research and development activities has at last been published, ready for its first reading at the next Research Council on 8 February. The new strategy has been under discussion for some two years, ever since Vicomte Etienne Davignon, the European Commissioner for Industry and Energy, added the research portfolio to his remit.

Ever since research became an accepted part of the EEC's activities in the mid-1970s, it has developed in a rather haphazard way. Today it presents a confusing mish-mash of different types of programmes, unevenly covering a growing number of fields and administered by unwieldy number of the Commission's directorate-generals. Some are "direct action" programmes, funded entirely out of the EEC's budget, others are "indirect action" programmes involving shared-cost contracts and the rest are "concerted action" programmes whereby national research is coordinated. And besides the Science, Research and Development Directorate-General (DG XII), four other directorate-generals share responsibility for these programmes.

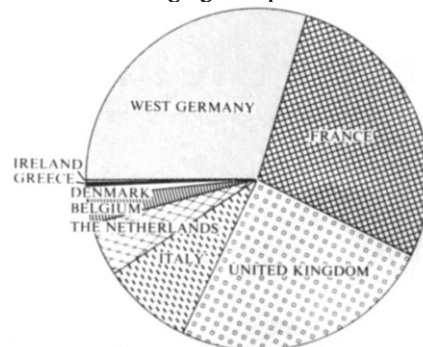
DG XII is still, however, a junior newcomer with a 2.6 per cent (1982) share of the EEC's budget and taking only 2.2 per cent of the total of publicly funded research in the EEC. The new strategy, which covers the years 1984-87, aims at bringing the first figure up to a modest 4 per cent, assuming a minimum outlay of 3,750 million European Currency Units (ECU) (\$3,787.8 million). Although, of course, the significance of a policy cannot be measured solely as a proportion of the total research spending it represents, there is also the multiplier effect of the shared-cost contracts and the political and economic impact of a coordination of national research policies. Above all, though, the Commission sees research and development as "a particularly effective means both of countering the effects of the present crisis and emerging from it".

The new framework is to be based on seven goals; promoting agricultural (130 million ECU) and industrial competitiveness, (1,060 million ECU) improving the management of raw materials (80 million ECU) and energy resources, (1,850 million ECU) reinforcing development aid, (150 million ECU) improving living and working conditions (270 million ECU) and lastly improving the efficacy of the Community's scientific and technical potential (297 million ECU). Each goal will have an action programme and a certain number of objectives which have been allocated a percentage of the budget.

The strategy relates not only to the way Community research and development

would be reorganized if the Ten's research ministers adopt it but would also represent a major shift of priorities. For instance, energy now takes 63.6 per cent of the total compared to the 18.46 per cent given to industrial competitiveness but the new framework would grant 1,060 million ECU to industry and 1,850 million ECU to energy.

Eventually Davignon hopes to move further down this road towards boosting the importance of research and development and changing the priorities of the



The distribution of the total sum of 23,842 million ECU spent by the governments of nine countries (Luxembourg excluded) on research, development and demonstration during 1981.

various goals. The Commission's paper itself raises two key questions about Davignon's strategy but answers only one of them — can the choices which have been made up to now be considered both adequate and appropriate in relation to the challenges which the Community must face in the 1980s?

The Commission's answer is, of course, a resounding "no". But the Ten's ministers must now be pondering the answer to the second question. Are the scale of the scientific and technological activities of the EEC adequate to the challenges facing the Community and its diverse activities and policies? And can they become so in future? **Jasper Becker**

Soviet satellite

Falling to Earth

RADIOACTIVE fallout from the nuclear reactor of the Soviet Union's disintegrating satellite Kosmos-1402 should not, at the worst, exceed the limits recommended by the International Commission on Radiological Protection, according to Academician Oleg M. Belotserkovskii, rector of the Moscow Physico-Technical Institute. He was taking part in a programme on Moscow television last Saturday which was obviously intended to allay public concern.

Kosmos-1402 was launched on 30 August 1982, with an orbital inclination of 65°, perigee 254 km, apogee 279 km, and a three-month ocean-surveillance pro-

gramme. Its power source, according to Belotserkovskii, was a uranium-235 reactor with a beryllium reflector shell. It also carried, he said, a safety system "in accordance with international recommendations" which would ensure dispersal of the radioactive material during burn-up, should the normal disposal procedure — insertion of the reactor into a high-altitude orbit — fail.

Kosmos-1402 ended its active life, said Belotserkovskii, on 28 December. The reactor separation system was triggered by the usual signal from the control centre, but the booster which should have transferred the reactor into an orbit at an altitude of at least 800 km apparently did not operate. The satellite remained — split into three parts — in a low-level orbit. One fragment re-entered almost immediately and burned up on 30 December, the main satellite structure is expected to re-enter at the end of January, and the reactor itself is due to re-enter in mid-February. Complete burn-up of the core during descent, and the consequent dispersal of the radioactive material in finely divided particles, is, Belotserkovskii assured his audience, "guaranteed".

Belotserkovskii's broadcast laid great stress on the fact that the United Nations Technical Subcommittee on the Peaceful Use of Outer Space had approved the use of nuclear-powered satellites, providing that appropriate safety measures were complied with — and implied that the Soviet nuclear power unit used in certain Kosmos satellites met with these requirements. (The designation "Kosmos" covers a wide range of scientific and military satellites, which can differ considerably in design and equipment. According to Western estimates, between 20 and 30 nuclear-powered Kosmos satellites have so far been flown.) Some US experts, however, have hinted that the design of these reactors is more than 15 years old, and under certain circumstances could spread hazardous radioactive material over a wide area — the type of hazard that the UN recommendations are specifically intended to prevent. Inevitably, the accident to Kosmos-1402 has recalled the "unscheduled re-entry" of the nuclear-powered Kosmos-954 which came down in north-west Canada just four years ago, although in that case the malfunction took place before reactor separation was attempted and the spacecraft re-entered as a single unit.

On that occasion, the search and clean-up operations involved 1,200 military personnel and close to 100 civilian experts and cost \$6 million (Canadian) of which the Soviet Union paid only half. Canada has already drawn up contingency plans for a similar search for the Kosmos-1402 reactor, including the use of airborne gamma ray spectrometers, although even if debris from the reactor should survive re-entry, there is only a 3 per cent chance that it would land in Canada. **Vera Rich**

Reagan Administration reshuffle

All-change in wake of Rostow

Washington

PRESIDENT Reagan fired his arms control chief, Eugene V. Rostow, last week as part of a shake-up of his Administration in arms control, health and conservation. Rostow is to be replaced by the 36-year-old former Pentagon official, Kenneth Adelman, who is now part of the US delegation to the United Nations, and close to conservatives around Washington. The President chose David Emery, a former congressman from Maine, to be deputy director of the Arms Control and Disarmament Agency (ACDA) under Adelman. In addition, Richard Starr, who had been US representative to the conventional force reductions talks, was replaced by Morton I. Abromowitz, a foreign service career officer. The White House had sought Starr's resignation because he seemed to have become excessively concerned with security.

Rostow had clashed with the White House over several issues, the most important being his view that the Administration should signal to the Soviet Union some "intelligent flexibility" in the Intermediate Range Nuclear Force



Rostow — hawk or dove?

Negotiations to resume in Geneva on 27 January. Soviet leader Yuri Andropov has indicated some willingness to cut the number of Soviet SS-20 missiles and the number of warheads aimed at Western Europe, but conservatives in Congress and the White House want the Administration to stick to its original demand — "zero option".

In addition, the White House last week dropped its support of Robert T. Grey, an ACDA official whom Rostow had fought long and hard to have confirmed as deputy director of the agency. Grey's nomination had been opposed by conservatives in Congress seeking to influence Rostow's handling of arms control issues.

In a farewell to his staff, Rostow quoted Talleyrand, saying he had violated the Frenchman's famous principle "*surtout, pas de zèle*". Noting that ACDA was demoralized when he took over in 1980, he said he was sorry if he had brought renewed turbulence to the agency.

In an unrelated move, the President

appointed former congresswoman Margaret Heckler, who represented a district in Massachusetts until her defeat last year, to become the new Secretary of Health and Human Services. Heckler replaces Richard Schweiker who announced he would leave, for financial reasons, to take a post connected with the life insurance industry.

Schweiker's huge department was the target of large budget cuts in domestic programmes, but he tended to protect the research community, including to some extent the National Institutes of Health.

Heckler represented Massachusetts for years, and might be expected to be sympathetic to the needs of the research community. In addition, she sat on the House of Representatives Science and Technology Committee. But one congressional source says that in the last Congress she took the opposite position from the research community — sometimes with a vengeance. She supported the animal rights bill prominently, she supported small business set-asides, which researchers argued would diminish their funding, and she supported conservatives seeking to limit fetal research.

A change of importance to environmental issues occurred when the Department of Energy (DoE) fired its top conservation official, Maxine Savitz, deputy assistant secretary for conservation and renewable energy. Savitz had been with DoE since its formation and had become an articulate spokesman for the programmes she came to head. DoE's conservation efforts have been a target of the Administration. **Deborah Shapley**

US universities

Biological sciences ranked

Washington

A TOTAL of 616 US biological sciences departments have been rated in the latest report of the Conference Board of Associated Research Councils, issued last week. The board, under the auspices of the National Academy of Sciences, is releasing its surveys volume by volume. Previous instalments dealt with the physical sciences, humanities and engineering (see *Nature* 299, 476; 1982). The last volume will cover social and behavioural sciences.

As in its other reports, the board declined to assign a single ranking to each department. Previous surveys that did so were criticized, especially by middle and low-ranked universities, for overlooking some of their good points.

Thus the Conference Board tried to be more subtle and asked respondents to rate

each department by 16 different measures, ranging from library size to the employment plans of graduates. Despite this effort, measure number eight — the scholarly quality of a department's faculty — has been seen as the key index to overall quality.

The survey divided biological sciences into six categories: biochemistry, botany, cellular/molecular biology, microbiology, physiology and zoology. Because work in these fields can occur in departments with various titles, the schools themselves were allowed to categorize their own departments. The top-ranked departments in two categories are indicated below, with the departments being ranked in parentheses.

Top ranked departments (scale 1-5)

Biochemistry	
MIT (Biochemistry)	5.0
Harvard (Biochemistry and Molecular Biology Graduate School)	4.9
Stanford (Biochemistry)	4.9
UC Berkeley (Biochemistry)	4.6
Univ. Wisconsin, Madison (Biochemistry)	4.6
Yale (Molecular Biophysics and Biochemistry, Pharmacology, Biology)	4.5
Cellular/molecular biology	
MIT (Biology)	4.9
Caltech (Biology)	4.8
Rockefeller (Cellular Biology, Molecular Biology)	4.8
Yale (Biology, Cell Biology/Molecular Biophysics and Biochemistry, Genetics)	4.7

Since biology has advanced rapidly in the past few years it is useful to know which departments have advanced the most:

Departments most improved in past 5 years (scale 0-2)

Biochemistry	
Univ. Texas San Antonio Health Science Center (Biochemistry)	1.7
UC San Francisco (Biochemistry)	1.7
Virginia Commonwealth University Medical College (Biochemistry)	1.7
Univ. Alabama, Birmingham (Biochemistry)	1.6
Univ. Texas, Houston Health Science Center, (Biochemistry and Molecular Biology, Biochemistry)	1.6
Cellular/molecular biology	
Univ. Utah, Salt Lake City (Biology)	1.8
UC Los Angeles (Molecular Biology)	1.6
Stanford (Structural Biology)	1.6
Syracuse Univ. (Biology)	1.6
Univ. Virginia (Biology)	1.6
Univ. North Carolina, Chapel Hill (Cell Biology, Molecular Biology)	1.6

As measured by the scholarly calibre of the faculty, the best microbiology programmes were at MIT and Rockefeller. In botany, the best were UC Davis and Vanderbilt. The best physiology programme was at UC San Francisco and the best zoology programme was at Harvard.

The report cautioned that the ratings for middle and lower ranked schools were less certain because fewer of the respondents were familiar with their programmes.

Deborah Shapley

Spina bifida trial

Research council goes public

PROFESSIONAL disagreements about the Medical Research Council's spina bifida trial were aired in public last week at a remarkable meeting in London called by the National Childbirth Trust. At the end of last year the council decided to go ahead with a double-blind trial of the efficacy of folic acid and other vitamin supplements in preventing recurrences of neural tube defects (NTD) in human births (see *Nature* 16 December, p.565).

Although last week's meeting seemed to agree that a trial of some kind should be held — the trust's view from the outset — representatives of the trust, including Professor J.H. Edwards (University of Oxford) and Professor Derek Bryce Smith (University of Reading), expressed concern about the levels of vitamin doses to be used, the form in which subjects' consent would be obtained and the criteria to be used for calling off the trial in midstream in the light of accumulated information.

As outlined by Professor Colin Dollery, chairman of MRC's systems board, and Dr Nicholas Wald, coordinator of the trial, a double-blind test involving some 2,000 women will be carried out over a period of five years. Mothers who have already had one NTD birth will be asked to participate by their physicians. If they consent to take part they will be given either one of three vitamin supplements or a control pill for at least 28 days before and 28 days after conception. Since it is impossible to determine when a woman will conceive, some women may be consuming supplementary vitamins for a period of years. The

identity of a woman's pill will only be known to a central monitoring committee; neither she nor her doctor will know.

It is this double-blind procedure that both distinguishes the MRC trial from those previously conducted (Smithalls, *Lancet* i, 339; 1980) and provides a bone of contention for critics of the trial. Is it ethically sound to withhold a possible treatment, whose effectiveness has been implied if not proved, in order to assess the results beyond reasonable doubt?

Adding fuel to the fire, the doses of folic acid that MRC proposes to administer are ten times greater than those given in previous tests, which were roughly at physiological levels. The reason given by MRC is that these levels will allow the monitoring of urine samples to ensure that supplements have been taken, but the effect of high folate doses is unknown. However, Dr Thomas Mead, chairman of the MRC clinical trials committee, assured last week's meeting that the trial would be halted should either any adverse effects or beneficial effects become apparent.

Since the decision to participate in the trial is clearly a major commitment, both representatives of MRC and their critics agreed over the need fully to inform putative parents before their consent is obtained. However, MRC's outline of the trial procedures does not specify that written consent is required, leaving that to the discretion of the regional centres. It seems likely that this point will be cleared up before plans are finalized.

There was also some concern last week

that a decision to enter the trial would not be unbiased but would be strongly influenced by the unique relationship of physicians with their patients. In a survey conducted by the National Childbirth Trust, women expressed a desire to comply with their doctors' requests, and a fear that failure to participate in the trial would result in substandard treatment during pregnancy. However, the majority of women said that they would start taking vitamin supplements independently of medical advice. In the light of this, it is difficult to see how MRC can adequately inform the subjects before obtaining their consent and still secure their participation.

Melanie Kee

UK parliament

Testing the waters

THE House of Lords Select Committee on Science and Technology, fresh from its largely unsuccessful attempt to improve the provision of scientific advice to the British government, has now added its voice to those who say that British water supplies and sewerage systems are in danger of catastrophic collapse unless more is spent on repair, renewal and research. The report, published last week (*The Water Industry*, HMSO, £6.00), appeared on the eve of the decision by workers in the water industry that they would promptly strike for more pay.

The explanation of the committee's choice of this unfashionable topic for a full-blown study is the transfer in 1973 of responsibility for research and national planning from central government to a number of constitutionally autonomous regional authorities. The committee says that this development has given central government, "especially the present", an opportunity to neglect its responsibilities.

Research spending as such seems to have been increasing more quickly than inflation in recent years, and is estimated at £20 million in the present financial year. Two-thirds of this is spent by the Water Research Centre, the cooperative research organization, supported by subscriptions from the water industry. Government departments responsible for the environment and agriculture each spend £2 million a year. This total does not include the substantial spending of water-related research by research councils and universities.

The committee says there is a danger that too little is being spent on research and that there is a high chance that too little attention is being given to what it calls strategic research — for example, the development of more accurate techniques for finding leaks in water pipes. It asks that the Department of the Environment should assume responsibility for planning a long-term research programme. ●

Einstein manuscripts on view

MANUSCRIPTS of Albert Einstein are to go on public exhibition for the first time in April 1985. Dr Reuven Yaron of the Hebrew University in Jerusalem said last week. The exhibition, together with a commemorative academic conference, will mark the thirtieth anniversary of Einstein's death, and will also focus on what the archivists of the National Library of Israel see as one of their most important acquisitions.

Under the terms of Einstein's will, his personal papers and manuscripts became part of a trust giving the trustees *carte blanche* to dispose of them, if they saw fit, for the good of the beneficiaries. All manuscripts and papers remaining when the trust was wound up were to pass to the National Library of Israel (housed in the Hebrew University), which Einstein himself helped to found. In the meantime, the manuscripts and papers were housed in the Institute of Advanced Studies at Princeton University in the United States, to which scholars had access only by special permission of the trustees. In 1977, following prolonged negotiations between the

trustees and Princeton University Press, Professor John Stachel began editing the papers and under the same agreement, a microfilm of the entire Einstein archives was deposited in the Princeton University Library.

In 1982, the trust was finally wound up, and a special committee, headed by Dr Yaron, was set up in Jerusalem to deal with the transfer. Moving the manuscripts to Jerusalem should not cause any major delay in the publication of the papers — Professor Stachel is now working from photocopies, and the first volume is due to go to press later this year.

For scholars, the transfer of the papers to Jerusalem should prove beneficial. In addition to the archive microfilm at Princeton, within a year scholars will have easy access to complete photocopies — or, if necessary, the originals — in Jerusalem. The Hebrew University has already entered into a tentative agreement with Princeton to prepare a *catalogue raisonné* of the manuscripts which should be completed within two years.

Vera Rich

Soviet oil prospecting

Lagging behind

SOVIET geologists prospecting in the oil and gas Tyumen' region of western Siberia are failing to keep up with the country's demands, according to Dr F. Salmanov, head of the Glavtyumen'geologiya prospecting organization. Writing in *Pravda*, Dr Salmanov has argued that by 1985, the Tyumen' geologists will have to double their work-load to keep up with the demands of the five-year plan, and by 1990 to treble it. But he acknowledges that they are hampered by the lack of technology appropriate to the geological and climatic conditions of the region.

This it seems, is why the Siberian branch of the Academy of Sciences of the USSR has now established a special council to deal with the development of the oil and gas industry in the Tyumen' oblast'. According to the chairman of the council, Dr Andrei A. Trofimuk, the council will concentrate on eliminating specific difficulties that beset the exploitation of remote deposits, and will propagate new techniques and working methods.

Several institutes of the Siberian branch have a special interest in oil and gas prospecting, including the institutes of geochemistry, mining, the Earth's crust, permafrost, and of geology and geophysics of which Dr Trofimuk is himself director. These institutes have already put forward proposals for speeding up prospecting, improving the transport of compressed gas and making trunk pipelines more reliable.

The new council will act as a coordinating body between the various institutes, and also between the research teams and the on-site workers. Members of the new council have already visited the oil and gas recovery sites at Novyi Urengoi, Nizhnevartovsk and Surgut, to tell the local production crews about the new research programme.

Research and new technologies are not, however, sufficient. Dr Salmanov cited the case of the high-performance TPS-172 drilling motor which gave excellent results in field trials during the Surgut survey of 1979, but which was then blocked by the Ministry of Chemical and Petroleum Machinery for no apparent reason. Ten of these machines were made available to Glavtyummn'geologiya in April 1982 and found to be successful yet the "Turbobor" production association which manufactures them is, says Dr Salmanov, "in no hurry" to expand production.

What is needed, Dr Salmanov suggests, is a totally new approach to the opening-up of western Siberia. The distribution of capital investment for research, machine building, prospecting and pilot projects should be reassessed. As things are, he complains, there is a tendency to economize by drilling fewer boreholes, so that surveys are delayed. He says that

special attention should be given to support services, including transport equipment and personnel, as well as to housing and social services in west Siberia.

The end-of-year statistics for the region suggest that much progress has in fact been made. In Urengoi alone, 100,000 square metres of accommodation was built last year, but Dr Salmanov complains that prospectors have benefited less than the existing inhabitants of the region.

At present, the west Siberian fields will remain the mainstay of the Soviet oil and

gas programme, with the exploitation of the reportedly-rich east Siberia fields left largely for the future. It is from Urengoi, in west Siberia, that the pipeline to Western Europe will draw its supplies. Dr Salmonov's criticisms and demands, (which appeared on the Party policy page of *Pravda*) coming so soon after the formation of the special oil and gas council of the Siberian branch of the academy, suggests that the Soviet Union is worried about the prospects of success in the region.

Vera Rich

French nuclear power

A year to watch new PWRs

WITH another seven nuclear power stations due to be connected to the French national grid in 1983, France's ambitious nuclear programme will soon be producing more than half the country's electricity. But the stations already in operation have had their problems (see table) and with such large numbers of similar, production-line reactors, the performance of the new stations this year will be keenly followed by those outside France favouring nuclear power.

France now has 22 working pressurized water reactors (PWRs), most of them less than 2 years old, but during the first 10 months of 1982 six of them operated at less than 25 per cent capacity, according to figures released by the national utility, Electricité de France (EDF). However, on the positive side, 10 of the reactors turned

in availabilities of more than 70 per cent, and one, Fessenheim 2, recorded 84 per cent.

The net availability of the whole system (including seven gas-graphite reactors and the Phénix fast breeder) was 50 per cent, according to published power-production figures. PWR availability alone was 51 per cent, against a planned figure of 62 per cent.

The shortfall will not recur in 1983, says EDF optimistically: the faulty control-rod tube-guide clips are to be replaced on all 22 reactors, involving shutdowns of "five to six weeks" on each, and problems with steam superheaters are "solved". So far there has been no trouble with the steam generators — corrosion of which plagued the Westinghouse reactors on which the French design is based. The French version is more rugged, the constructors claim.

Robert Walgate

The French nuclear power programme

	MW (electric)	Construction began	Connected to grid		MW (electric)	Construction began	Connected to grid
From 1954 to 1968				Second 900MW series			
Marcoule G-1*	2	1954	1956	(cont.)			
Marcoule G-2*	38	1955	1959	Le Blayais-3.....	920	1977	1983
Marcoule G-3.....	38	1956	1960	Le Blayais-4.....	920	1978	1983
Chinon A-1*	70	1957	1964	Cruas-1.....	880	1978	1984
Chinon A-2.....	210	1958	1965	Cruas-2.....	880	1973	1984
Chinon A-3	480	1961	1966	Cruas-3.....	880	1979	1984
Chooz A-1.....	310	1962	1970	Cruas-4.....	880	1979	1985
Monts d'Arrée.....	70	1962	1966	Gravelines C-5.....	920	1979	1985
St-Laurent A-1.....	480	1963	1969	Gravelines C-6.....	920	1980	1986
Bugey-1.....	540	1965	1972	Chinon B-3.....	870	1981	1986
St-Laurent A-2	515	1966	1971	Chinon B-4†	870	1982	1987
Phénix (breeder).....	233	1968	1973				
1970 Programme				First 1,300 MW series			
Fessenheim-1§	880	1970	1977	Paluel-1.....	1,285	1977	1983
Fessenheim-2†	880	1971	1977	Paluel-2.....	1,285	1977	1983
Bugey-2§	920	1972	1978	Paluel-3.....	1,285	1978	1983
Bugey-3.....	920	1972	1978	St-Maurice-1.....	1,285	1979	1985
Bugey-4 §	900	1973	1979	Flamanville-1.....	1,285	1979	1985
Bugey-5.....	900	1974	1979	Paluel-4.....	1,285	1980	1986
First 900 MW series				St-Maurice-2.....	1,285	1980	1986
Tricastin-1.....	920	1974	1980	Flamanville-2.....	1,285	1980	1986
Gravelines B-III.....	920	1974	1980				
Dampierre-1†	900	1974	1980	Superphénix			
Tricastin-2†	920	1974	1980	(fast breeder)			
Gravelines B-2†	920	1975	1980	Creys-Malville.....	1,200	1976	1984
Dampierre-2.....	900	1975	1980				
Tricastin-3.....	920	1975	1981	Second 1,300 MW series			
Gravelines B-3.....	920	1975	1980	Cattenom-1.....	1,275	1979	1986
Dampierre-3.....	900	1975	1981	Cattenom-2.....	1,275	1980	1986
Tricastin-4.....	920	1975	1982	Belleville-1.....	1,275	1981	1987
Gravelines B-4.....	920	1976	1981	Nogent-1.....	1,275	1981	1987
St-Laurent B-1§	800	1976	1981	Belleville-2.....	1,275	1981	1987
Dampierre-4.....	900	1976	1981	Cattenom-3†	1,275	1982	1988
St-Laurent B-2§	880	1976	1981	Nogent-2†	1,275	1982	1988
Le Blayais-1.....	920	1976	1981	Golfch-1.....	1,275	1982	1988
Le Blayais-2	920	1977	1982				
Second 900 MW series				Further 1,300 MW stations			
Chinon B-1.....	870	1977	1983	Chooz B-1†	1,275	1982	1988
Chinon B-2.....	870	1977	1983	Penly-1†	1,275	1983	1989

* Stations closed down

† 1982 and 1983 orders placed in October 1981

‡ Cracks detected in pressure vessel

§ Stopped in 1982 with tube guide or superheater problems

|| Availability below 25% in first 10 months of 1982

Open government in research

SIR — The Medical Research Council (MRC), in appearing to prevent persons receiving its support from taking part in a debate on a matter of scientific uncertainty and public concern, has done itself a disservice and lost public sympathy by implying that the scientific, social and ethical bases of its operations are not matters for public scrutiny.

But what can be done by researchers (scientific or medical) to ensure in such circumstances that the public interest is properly served? We must take as our premise that the public interest requires full and unfettered discussion of all the issues. Clearly an employer threatening, however covertly, the livelihood or scientific career of anyone expressing dissenting views is acting improperly. Nevertheless it is the whole scientific community which is to be blamed if the individual sets those factors higher than public conscience, scientific integrity and professional standards.

In this particular instance, involving the merit of clinical trials to establish the efficacy of folic acid supplement in reducing the risk of spina bifida, MRC — an organ of public policy — is going directly against standards of conduct explicitly adopted for governmental organizations by this country and set out in "Recommendation to Member States on the Status of Scientific Researchers" agreed by the General Assembly of UNESCO in 1974. In this at Paragraph 37 we find: "Member States should, in consultation with scientific researchers' organizations and as a matter of standard practice, encourage the employers of scientific researchers, and themselves as employers seek: (a) to regard it as the norm that scientific researchers be at liberty and encouraged to publish the results of their work; (b) to minimize the restrictions placed upon scientific researchers' right to publish their findings, consistent with public interest". Further, at Paragraph 34: "... Member States should seek to ensure that scientific researchers may: (a) receive without hindrance the questions, criticisms and suggestions addressed to them by their colleagues ... as well as the intellectual stimulus afforded by such communications and the exchanges to which they give rise". In even more specific vein the 1979 Annual Report of the American Association for the Advancement of Science's Committee on Scientific Freedom and Responsibility: "... silencing dissenters as bearers of ill-tidings may seem in the short term to be the simplest way to deal with the difficult problems which they raise. But in the longer term enhancing the professionalism of scientists and engineers, and defence of their integrity when following its dictates, are vital to the welfare of our entire society" (p. 65).

In the dynamic tension of a pluralistic scientific enterprise responsive to socio-political priorities one key force is evidently

missing from the United Kingdom. This is a body for researchers capable of formulating, expressing and upholding by force of consensus professional standards in research. Such standards should be upheld both by expressing disapproval of bad practice and by support of those who are pressured towards bad practice. It is a long-term objective of my association to create such a body.

J.P. DICKINSON
(Chairman)

The Association of Researchers in
Medicine and Science Ltd, Leeds, UK

Folic acid deficiency

SIR — Discussion of folic acid deficiency and spina bifida (Nature 300, 302-310, 396; 1982) provokes the comment that the existence of folic acid was first uncovered by the findings of Lucy Wills¹, a British physician, that pregnant women in India developed a deficiency on inadequate diets. Following the synthesis of folic acid², the requirement for it was shown to be increased by pregnancy, and deficiencies of folic acid in pregnant women are regarded as worldwide in scope. "Natural" sources of folic acid, because they usually exist as conjugates, are often less biologically available than the synthetic product. An added impetus to providing pregnant women with folic acid came when Hogan and co-workers³ found hydrocephalus in rats born to mothers on a diet deficient in folic acid, and Nelson et al.⁴ reported that multiple congenital abnormalities in young rats were produced by maternal folic acid deficiency during gestation. There is no need for overdosage, and the recommended daily allowance (in the United States) for folic acid in pregnancy is 0.8 mg, corresponding to a bulk folic acid cost of less than one-tenth of one cent.

Introduction of folic acid in vitamin products was strongly opposed by some haematologists because large doses given to patients with pernicious anaemia in relapse allowed their neurological symptoms to progress while haematological remission took place. The US Food and Drug Administration maintained restrictions on folic acid for many years because of this. The neurological symptoms did not occur if vitamin B₁₂ was administered simultaneously⁵. The biochemical aetiology of this effect depends on the interrelationship of folic acid and vitamin B₁₂ in methyl group synthesis and transfer⁶. Aggravation of neurological symptoms by large doses of folic acid could conceivably occur in a pregnant woman with pernicious anaemia if she were not given vitamin B₁₂, but this concatenation of circumstances should be rare. In contrast, it is likely that many pregnant women have been deprived of their need for folic acid, which is at

borderline levels in many diets, by this caveat.

It is encouraging to read, at long last, that Members of Parliament are asking why their constituents are being "denied an apparently effective treatment", including folic acid. Whether folic acid deficiency is responsible for some cases of spina bifida is not clear, but it seems obvious that pregnant women should receive their requirement for folic acid.

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Soviet mathematicians

SIR — I should like to comment briefly on the decline in the number of citations to the Soviet mathematical literature by US mathematicians, noted on p.568 of your issue of 14 October 1982. The explanation is closer to hand, and simpler, than your writer suggests.

Beginning in the late 1960s, Soviet discrimination against political undesirables and Jews has been particularly blatant in the field of mathematics. Students whose parents fall into either one of these categories were denied admission to the best mathematics departments in the Soviet Union, and are currently prevented from attending university at all. Students whose background is considered undesirable and Jewish students, who managed to obtain undergraduate degrees, have encountered increasing difficulty in obtaining graduate degrees. Many mature mathematicians have been prevented from publishing in the more prestigious Russian mathematical journals and even from publishing at all. The journal *Matematicheskii Sbornik* is the worst offender in this regard.

These policies of the Soviet government have led a substantial number of the best Russian mathematicians to leave the Soviet Union. This, in addition to the policies described above, has resulted in a serious deterioration of the Soviet mathematical literature, which, of course, accounts for the drop in the number of citations.

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Darwin's blood group

In the correspondence "Grouping Darwin" (Nature 300, 573; 1982), the following corrections should be made to the table of likelihoods: for 43 read 34, for 19 read 18.

Storm in teacup about Poynting vector

An argument about the ambiguity of the standard expression for energy-flux in the electromagnetic field asks too much of physics.

REFORM is an uncertain business, as Professor C. S. Lai must by now have learned from his attempt two years ago to modernize, even improve upon, the standard expression for the transport of energy by an electromagnetic field. It is now not quite a century since the literature was first adorned with J.H. Poynting's succinct statement of the energy flux of an electromagnetic field

$$\mathbf{S} = \mathbf{E} \times \mathbf{H}$$

where $\mathbf{E}$ and $\mathbf{H}$ are the vector fields specifying the electric and magnetic field strengths and the symbol connecting them indicates the usual vector product, itself a vector field. For the best part of a century, the Poynting vector has seemed to enjoy a great many advantages, not the least of which is that, in the plane-wave solution of Maxwell's electromagnetic wave equation, $\mathbf{E}$ and $\mathbf{H}$ are plainly perpendicular to each other and to the direction in which the wave is travelling and, given the phase relationships between the electric and magnetic vibrations, the energy-flux density at any point works out as a constant vector in a fixed direction.

Reform

Lai's programme of reform for the Poynting vector, first put forward in the *American Journal of Physics* (49, 841; 1981) starts from the simple observation that the revered Poynting vector yields an awkward non-zero result when some pattern of entirely static electric and magnetic fields is slotted into the equations. To be sure, the quantities concerned, $\mathbf{E}$ and $\mathbf{H}$, are at some level related by Maxwell's equations to each other and to the patterns of moving electric charges (currents) that there may be, but it seems on the face of things to be offensive that static field should yield non-zero energy fluxes. So why not find some other way of constructing an energy-flux vector that will tie in with Poynting for the obviously crucial plane-wave solution of a vibrating electromagnetic field but otherwise also yield a zero energy flux for a pattern of static fields? That was Lai's programme two years ago.

Formally, the solution is quite straightforward. Given the relationships between the electric and magnetic field strengths, $\mathbf{E}$ and $\mathbf{H}$, and the electromagnetic vector potential $\mathbf{A}$ and the electrostatic potential ϕ , the definition of the electric current $\mathbf{J}$

and Maxwell's equations, it turns out that it is always possible to add to the vector field represented by the Poynting vector the expression $\text{curl}(\phi\mathbf{H})$ or $\nabla\Lambda(\phi\mathbf{H})$. In many physical respects, the two vector fields are the equivalents of each other, for the train of argument that leads to Gauss's theorem also shows that the integral of the added vector field over the surface of a closed volume (with an appropriate definition of the scalar product of the vector field at the surface and the normal to the unit of surface area) is identically zero. So the modified Poynting vector yields the same description of the transfer of power within the field, but plainly the modified vector can also, by suitable manipulation, also be set equal to zero in those parts of the field in which the electric and magnetic vectors themselves are static.

So is that not a great advantage? The vector field describing the energy flux in an electromagnetic field can be given the desirable property that when the fields are static, unchanging in the course of time, no energy flux need be involved. This feature, among others, seems to have been one of Lai's boasts two years ago. In the most recent issue of the *American Journal of Physics* (50, 1162, 1165 and 1166; 1982), however, at least three critics take him to task for having overlooked a simple point, the fact that the original form of the Poynting vector is at least unambiguously defined by the electric and magnetic field strengths but that the modified version of the vector, including as it does a term dependent on the vector and scalar potentials of the electromagnetic field, is not unambiguous but, rather, dependent on a suitable gauge.

To put the difficulty another way, just as a scalar potential may always without physical consequences be modified by the addition or subtraction of some constant, so a vector potential such as $\mathbf{A}$ may always be modified, without physical consequence, by the addition of a vector field defined everywhere as the gradient of some scalar function.

So, the problem with the Poynting vector is, on the face of things, a simple question of choosing between one set of ambiguities and another — an energy flux vector field that yields a non-zero energy flux even when the fields are static, and a modified vector field which avoids the second difficulty but at the cost of yielding ambiguous expressions for the energy flux within an electromagnetic field except

when the gauge (the scalar function whose gradient is added arbitrarily to the vector potential) is chosen suitably.

In the long history of this subject, now almost a century old, issues like these have been debated frequently and often fiercely. So what is, or should be, the correct form of a vector such as the Poynting vector, describing the energy flux within an electromagnetic field? The simple answer is simply to insist, as common sense requires, that if the field is static, the transport of energy should be zero. If only the expectations of common sense more often had the force of law.

Identity

For the identification of the classical Poynting vector with the energy-flux within an electromagnetic field is, in its essence, a generalization of some kind. Physically, the simplest thing to say is that the transport of energy across some closed surface enclosing a small volume will be related to the change of the amount of energy closed within that volume. In the electromagnetic field, the internal energy of the field is determined by the scalars, quantities that represent the squares of the field strengths, and however the scalar and vector gauges are chosen, the calculation of the changes of energy content are entirely unambiguous.

Ernst Mach would have a simple comment on this storm in a teacup — always relate arguments about the meaning of physical qualities to calculations of quantities that can be measured. For what it is worth, Lai's discussion of alternatives to the Poynting vector is an interesting way of getting at the ambiguity introduced by the concept of gauge in the electromagnetic field.

The important point is merely that the ambiguities to which Lai and his three critics have drawn attention are neither unexpected nor physically meaningful. Just as, in a world in which the only attributes of numbers are their magnitudes (strictly, moduli), it makes no difference whether all numbers are multiplied by some complex number of unit modulus, so it is right and proper that the functions of position and time from which electric and magnetic field strengths are derived by differentiation should be within limits arbitrary. If they were not, it would not be possible to satisfy Mach's positivist criterion that all supposedly physical quantities should be unambiguous. □

Sequence banks

Searching for sequence similarities

from Temple F. Smith and Christian Burks

WITH the recent establishment of nucleic acid sequence data bases in both Europe¹ and the United States², highly organized and nearly complete listings of the known experimentally determined sequences are now available for exhaustive computer-assisted sequence comparisons. As several recent papers and a workshop* on the computational analysis of DNA sequences make clear, it now seems practicable to carry out global searches of the data for functional, structural and evolutionary relationships between sequences. Initial searches carried out at the National Institutes of Health and Los Alamos National Laboratory have brought to light several fascinating potential relationships, including at least one intra-sequence repeat that may represent a misadventure in cDNA cloning.

Three problems associated with such global searches were discussed at the meeting: increasing their efficiency, confirming their mathematical rigour and/or reliability and, finally, determining the statistical significance of the identified similarities. While there exists no known complete analytical solution of the last problem, the large size of the data bases provides for empirical analysis of the significance of any particular result.

The first two questions are interrelated, and two different approaches to addressing them were reported. D. Lipman and W. Wilbur (NIH) have developed a fast heuristic algorithm³, while at Los Alamos National Laboratory a rigorous generalization⁴ of the Needleman-Wunsch algorithm has been used. The former approach results in impressively fast searches on a medium-sized computer with reasonable recognition of sequence similarities. In contrast, although the Needleman-Wunsch algorithm is better understood, it requires access to a very large computer to achieve reasonable speeds (44,000 comparisons of all pairs of vertebrate sequences among themselves required approximately 170 min on a CRAY).

A number of very interesting similarities have been identified using the currently implemented local algorithm⁴ on the vertebrate portion of the NIH Nucleic Acid Sequence Data Bank² at Los Alamos. First, all the obvious homologies among such well studied gene families as the haemoglobins are identified with similarity values from three to twenty 'standard deviations' above the mean similarity value. It should be noted, though, that standard deviations have to be derived

empirically because distributions of similarity values obtained for the maximally similar local subsequences are rarely normal. However, such a simple measure of an outlier's relative significance is, no doubt, still useful.

A complete investigation of the similarities between the vertebrate sequences and the members of the Alu/B1/4.5S middle repetitive sequences reveals not only the known homologies but also examples of a B1-like sequence within a mouse immunoglobulin κ chain on the complement strand and in a mouse histone gene. A global search using such a dispersed family is instructive in that known family member similarities are often just barely distinguishable from apparent chance similarities within the large data set used. On the other hand, what appears as unquestionably significant similarities between the whitefish antifreeze protein and the spacer between the *Xenopus laevis* 5.8S and 23S rRNA and numerous other known sequences indicates only similarity between the high CC(purine)CC repeat in the fish and other very C- or G-rich regions. Weak similarities supporting previously noted homologies at the amino acid level between such 'unrelated' proteins as the chick ovalbumin and the primate α_1 -antitrypsins⁵ were easily identified. Similarities were also found between other unrelated sequences such as the *X. laevis* rRNAs and a herpes simplex virus transcript intron, as well as between the anglerfish preproinsulin and a mouse immunoglobulin heavy chain.

Finally, the importance of these global searches showed up most strikingly when comparisons involved the complementary strand sequence. Here several cases were found where the most similar sequence was its own complement. While this is not surprising for structural RNAs considering their secondary structure requirements, it is unusual for protein-coding regions. One case has been found just outside of the 3' end of the human preproinsulin transcription region. This is an imperfect inverted repeat of approximately 150 bases.

Even more interesting is the perfect 113-base inverted repeat in the human leu-enkephalin precursor cDNA⁶. Consideration of the position of this particular inverted repeat (the initial 113 bases and 725 bases in the coding region) suggests that it may simply be a 'hooking error' — that is, the reverse transcriptase that was used to backtranslate the RNA self-primed when it reached the end of the sequence and worked back along the sequence a short distance. Further

investigation is needed, particularly since homologous sequences are known in several other organisms that could result from similar errors.

In assessing the statistical significance of potential similarities, one must consider whether the disproportionate representation of some out of all biologically realizable DNA sequences in current data bases might not impose a bias on any evaluation of significance. There is a natural tendency for biochemical research to gravitate towards species and genes for which the genetics and biochemistry are known. Though this and similar prejudices may bias the apparent centre of distribution of chance similarities, the range of the bias from comparison to comparison is small relative to the width of the 'chance distribution' itself and thus has only a slight influence on the empirical identification of significant outliers.

W. Goad and M. Kanehisa have developed a heuristic approach⁷ to the quantitative estimation of the significance of the sequence similarities; the current popular approach is to use Monte Carlo simulations. Higher-level correlations, such as the known domain-dependent base nearest-neighbour frequencies (as suggested by W. Fitch⁸), or even the recognized codon usage correlations⁹, should readily be included in the latter. In any case, to date, the empirical comparisons with the known biologically realizable sequence distributions appear to be very conservative means of estimating statistical significance.

It will shortly become routine for each new entry in the US nucleic acid data bank, GenBank¹⁰, to be compared with all previous entries, and this should bring to light numerous new relationships. However, as noted by A. Maxam at the Aspen meeting, and so clearly reinforced by the recent work on various DNA-binding proteins^{11,12}, functional sequence similarity may not be adequately reflected in just the simple base-specific comparisons. Rather, comparative searches must be based, in addition, on equivalent local twist and chemical sites. □

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*The workshop was held at the Aspen Center for Physics, Aspen, Colorado.

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Coffee and opiate receptors

Another cup of coffee?

from Leslie L. Iverson

As writers, artists, scientists and anyone else who has to meet a deadline knows, the mild stimulant effects of coffee and even its ability to provoke anxiety and insomnia can be helpful. We all know that the stimulant effects of coffee are due to caffeine, and that this acts by inhibiting the enzyme cyclic nucleotide phosphodiesterase, thus enhancing the various effects mediated by this ubiquitous intracellular second messenger. Or that was what we thought we knew. In this issue of *Nature*, Boublik *et al.*¹ (p.246) report that coffee contains another potentially important psychoactive substance, and other recent results call for a radical change in our views of how caffeine and related xanthines act.

Boublik and colleagues report the startling discovery that solutions of instant coffee powder, both normal and decaffeinated, are able to inhibit the *in vitro* binding of ³H-labelled naloxone and other ligands to opiate receptor sites in brain membranes. The active substance responsible for such effects can be extracted with ether, is of low molecular weight (1–3,500), dialysable, heat stable and resistant to peptidase degradation. The unknown material is currently being purified and its chemical identity will be awaited eagerly; it is clearly not caffeine, which is inactive at opiate sites. The partially purified material acted as an opiate antagonist, blocking the effects of morphine in the guinea pig ileum *in vitro*. The novel opiate material was present both in instant coffees and in coffee brewed from freshly roasted beans, but was not detectable in tea or in various other foods. Even more remarkable is that the opiate antagonist is present in substantial quantities; Boublik *et al.* estimate the concentration in a normal cup of coffee to be about five times higher than the IC₅₀ for ³H-naloxone displacement *in vitro*.

The question of how this might affect the coffee drinker has yet to be addressed. It is possible that the active substance is not absorbed or is rapidly metabolized, and thus never gains access to the nervous system. On the other hand, opiate antagonists such as naloxone have few if any detectable effects in normal subjects. When given in a blind manner, normal subjects are unable to determine whether they have received naloxone or placebo². It is thus possible that coffee drinkers are unwittingly exposed to opiate receptor blockade, although it is not clear how this may contribute to the overall effect.

It remains more likely that the stimulant actions of coffee do result from the substantial amounts of caffeine consumed — about 100 mg per cup. Recent findings point to cellular receptors for adenosine,

rather than phosphodiesterase, as a likely target for caffeine and other psychoactive xanthines^{3,4}. It has been known for some time that caffeine and related methyl xanthines (for example, theophylline) can antagonize the effects of adenosine on a variety of tissues, and this now seems a more likely mechanism of action than the very weak inhibitory effects which these compounds exert on cyclic nucleotide phosphodiesterase. There is increasing interest in the possible role of adenosine as a modulator of various cellular responses to hormones and to neurotransmitters^{5,6} as part of a general focus of interest on purines as possible chemical messengers or modulators⁷.

Adenosine receptors in brain membranes have recently been identified by *in vitro* binding techniques, using the stable analogues 2-chloroadenosine⁸ or N⁶-cyclohexyladenosine⁹ as radioligands. The latter compound proved to be a potent behavioural depressant when administered to mice¹⁰, and a good correlation was found between the potency of caffeine and other xanthines to stimulate behavioural activity and their potency in inhibiting the binding of ³H-cyclohexyladenosine to brain mem-

branes *in vitro*.

Both lines of research on the psychopharmacology of coffee raise many unanswered questions. The possibility that coffee drinkers regularly imbibe an opiate receptor antagonist reminds us of how little we understand of the normal functioning of the endogenous opioid mechanisms. Does the lack of effect of opiate antagonists in normal subjects mean that these systems are used only *in extremis* — in conditions of stress or pain? If caffeine acts by blockade of adenosine receptors in brain, what function do such receptors normally have, and where does the adenosine come from? In seeking the answers to such questions many more cups of coffee will certainly be needed. □

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Pacific volcano statistics

How many ocean volcanoes?

from Peter J. Smith

GEOLOGISTS have known for decades that there are thousands of dead and alive volcanoes on the ocean floors, but they have never been too sure what to make of most of them. The few volcanic islands and seamounts in linear formation and with an age progression along each chain have recently attracted attention as 'hotspot volcanoes' and have become vaguely assimilated into the plate tectonic story; but the majority, apparently unrelated individual features, have hitherto often been regarded as of little general significance. It is probably fair to say that the volcanic pimpling of the oceanic lithosphere has been seen largely as a curiosity with only marginal, if any, relevance to global tectonics.

But that view may be quite wrong, as a simple statistic derived by Batiza suggests (*Earth planet. Sci. Lett.* **60**, 195; 1982). Batiza has examined all 12,000 seamounts and volcanic islands shown on bathymetric charts covering most of the Pacific and concludes that they account for about 5 per cent by volume of the oceanic volcanic layer. Moreover, bearing in mind that the bathymetry of much of the Pacific floor is

not fully known and extrapolating from those areas surveyed in detail, it becomes clear that the true number of volcanoes in the Pacific could be as high as 55,000. In other words, anything up to 25 per cent of the oceanic lithosphere could be accounted for not by the near-horizontal sheet usually envisaged but by the volcanic features superimposed upon it.

That rather changes the conventional perception of seamounts' importance, for 25 per cent is no insignificant proportion. On the other hand, the statistics on current activity are rather less impressive. Batiza estimates that about 3 per cent of oceanic volcanoes are active, accounting for only about 4 per cent of the total volcanic production from seamounts and oceanic ridges together. Unfortunately, little is known about the internal compositions of seamounts because drilling has not completely penetrated one; but if the chemistry of seamounts turns out to be vastly different from that of ridge-generated material, even the 4 per cent that oceanic volcanoes represent could have a profound influence on bulk lithospheric composition.

So with the possibility in mind that a

greater knowledge of oceanic volcanoes might put important constraints on geochemical and geophysical models of the lithosphere's evolution, Batiza has looked more closely at some of the other seamount statistics. Thus he finds, for example, that in the Pacific as a whole, and especially in the North Pacific, there is a monotonic increase in the density of non-hotspot seamounts (number of seamounts per unit area of ocean floor) going back to the Eocene (about 37–53 million years ago). If all oceanic volcanoes had been produced at ridge crests and had spread with the lithosphere to their present positions, this would mean that since the Eocene there has been a gradual reduction in the rate of production of oceanic volcanoes. But it would also mean that each volcano would be of roughly the same age as the lithosphere upon which it sits, which is known not to be the case. The more likely explanation, therefore, is that new volcanoes can erupt on lithosphere of any age and that the density of seamounts increases into the past simply because the older lithosphere has been around longer.

In which case, what causes non-hotspot volcanoes to occur away from the ridges? Unfortunately, there appears to be no strong evidence to suggest that non-hotspot volcanoes are produced any differently from recognizable hotspot volcanoes, which means that they could well be the result of deep-mantle plumes that do not live long enough to give rise to volcanic chains as the lithosphere moves over them. On the other hand, to the extent that volcanic chains have been attributed to propagating fractures in the lithosphere as an alternative to the mantle plume hypothesis, non-hotspot volcanoes could just as well arise where lithospheric fractures provide a conduit for the transmission of magma from the asthenosphere below. There is, in fact, some evidence to suggest that oceanic volcanoes are more abundant where lithospheric fracturing is more frequent, but the correlation does not yet preclude plumes. Hotspot and non-hotspot volcanoes may or may not have distinct origins; it is too soon to say.

But to get back to statistics: although there is an increase in seamount density back to the Eocene, there is then a decrease in density going back further from the Eocene to the late Jurassic (about 136–157 million years ago). This is surprising at first sight, for the explanation of the increase during the more recent period must surely also be relevant to the earlier period, and there is no obvious reason why the Eocene should have been singled out for the favour of a seamount density maximum. The cause lies partly with the sediment that accumulates on the volcanic lithosphere and partly with the lithosphere itself.

With increasing age of the oceanic lithosphere there is a gradual decrease in the proportion of the smaller volcanoes observed and a corresponding increase in

the proportion of larger ones. The reason is partly that as the sediment gets thicker it submerges the smaller volcanoes, which therefore become no longer detectable. At the same time, the older and hence the thicker the lithosphere (because of cooling), the greater is the maximum volcano size the lithosphere can support. Thus although there is, beyond the Eocene, a decrease in the number of seamounts per unit area, the average size of the seamounts increases. Moreover, in terms of overall volume, the increase in average size more than compensates for the loss of numbers. The aggregate volume of seamounts per unit area thus increases with age from the present to the Jurassic with a particularly dramatic increase (by a factor of about 7) between the Eocene and the Jurassic.

There seems little doubt, then, that most of the individual volcanoes now observed on the ocean floors erupted away from the

ridges, for Batiza has adduced strong quantitative evidence that this is the case. By contrast, statistics have been unable to demonstrate that there is any difference between the factors controlling the production of non-hotspot volcanoes and those governing the generation of hotspot volcanoes, although this is far from saying that no such difference exists. To this extent Batiza's analysis has provided less geophysical insight into the workings of the lithosphere than he would perhaps have wished. The geochemical implications of seamounts are not yet much clearer either; but at least it can be said, on the grounds of volume alone, that the geochemical consequences of the existence of seamounts are unlikely to be insignificant.

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Onco gen

What has moved into c-mos?

from Peter Newmark

TWO separate points are worth making this week. The first is to draw attention to the paper beginning on page 262 of this issue of *Nature* in which Nicholas Gay and John Walker point out a strong homology between a probable ATP-binding sequence of the β -subunit of mitochondrial ATPase and the amino terminus of the p21 protein that is encoded by *c-ras*^H, the cellular oncogene equivalent of the Harvey murine sarcoma virus transforming gene. It is within the amino terminus that a single amino acid change has been implicated in a human bladder carcinoma.

For the second point I am grateful to Nobumichi Hozumi and Robert Hawley of the Ontario Cancer Institute who have spotted a very interesting sequence homology, related to the *c-mos* gene that had apparently been activated in a mouse myeloma by the replacement of one of its ends by a movable genetic element (Rechavi *et al.* *Nature* 300, 582; 1982).

Rechavi *et al.* appeared to have identified a 159-base pair sequence that bore many of the hallmarks of the bacterial insertion sequence type of movable genetic element. They plumped for that interpretation because they detected no long terminal repeats, one of the characteristics of retrovirus-like movable elements.

But now it is possible to unearth strong evidence of a long terminal repeat in the Rechavi *et al.* sequence on account of a paper that Hozumi and Hawley, together with Marc Shulman of the University of Toronto and Edward Kuff, Anita Feenstra, Kira Lueders and Leonard Smith of NCI Bethesda, have in press in *Proceedings of the National Academy of Sciences*.

That paper is a follow-up to one by Hawley *et al.* in the same journal (79, 7425;

1982) in which it was shown that the immunoglobulin κ light-chain gene of two hybridoma cell lines that synthesized little of the light chain, contained retrovirus-like sequences within an intron. Restriction enzyme maps suggested that these sequences were intracisternal A particle genes, which are normally present in about 1,000 copies in the mouse genome.

Kuff *et al.* have now confirmed this suggestion and have sequenced the long terminal repeats of one of the sequences inserted into a κ light-chain gene intron.

Remarkably, the 338-base long terminal repeat sequences — which are almost identical — have greater than 90 per cent homology with 350 of the bases that have replaced the end of the *c-mos* gene in the myeloma of Rechavi *et al.* (the discrepancy in numbers is accounted for by a few minor insertions or deletions). The homology is only apparent when the myeloma sequence is read backwards from the junction with *c-mos* and converted into the complementary strand sequence. Then it is clear that it begins and ends with the same 4-base pair inverted repeat as the long terminal repeat of the intracisternal A particle gene and shares with it some other characteristics of long terminal repeats.

Therefore, an alternative interpretation to that of Rechavi *et al.* for what went on in the myeloma is that an intracisternal A particle gene became inserted in the opposite direction to *c-mos*, displacing part of it and, conceivably, activating what was left by a process similar to the activation of *c-myc* by avian lymphoid leukaemia virus in chicken B-cell lymphomas (Hayward *et al.* *Nature* 290, 475; 1981). □

Peter Newmark is Deputy Editor of *Nature*.

Dürer and geometry

Symmetry in an enigma

from Philip C. Ritterbush

THE geometric identity of a polyhedron figured in Albrecht Dürer's *Melencolia I* has eluded art scholars and even some scientists. In actual fact, it is an acute rhombohedron truncated on its axis of symmetry, suggesting that as early as 1514 Dürer appreciated symmetry relations.

According to interpreters of its pictorial symbolism, Albrecht Dürer's copperplate engraving *Melencolia I* (Fig. 1) portrays the intellectual enterprise of geometry at a time when it was emerging as a science from its antecedents in medieval philosophy. The mood of depression shown in the work reflects a mind's struggle to clear a mystery beyond its reach, posed by an angular block of stone. The geometric identity of that body is a puzzle that has pained scholars as much as it seems to perturb the geometrical muse in the engraving.

The historian of science Max Steck, who

surveyed Dürer's contributions to the theory of perspective and the development of regular solids from plane figures, found him 'an original and creative mathematician'¹. Notwithstanding this estimate of Dürer's geometrical ability, Steck concluded that the *Melencolia* body is 'not described in the mathematical literature and has no special properties'. Its form lay 'outside the field of classical geometry'. It was the artist's 'free invention'².

Others have taken an opposite opinion, that the figure either represents or might be derived from a perfect Platonic solid. One writer, who probably preferred books to geometry, identified it as a dodecahedron³. Wölfflin⁴ considered it possible that the figure was a cube from which two opposing summits had been removed (suggesting quaintly that the hammer lying by its side might have been used to knock them off).

Some art historians continue to regard the cube as its basic form⁵. Dürer's first draft of the figure was reproduced in a recent edition of his sketchbook with the caption, 'a truncated cube on a pedestal'⁶.

Erwin Panofsky, a noted authority on the historical context for Dürer's artistic ideas, thought the figure was derived from the octahedron and could represent a cube only if the pictorial space had been greatly distorted. The mode of perspective employed by Dürer was not as 'scientific' as it later became⁷. Despite this shortcoming, Panofsky deemed Dürer's abilities adequate to portray the geometrical properties of a cube had he intended to do so. 'It would do injustice to Dürer's power of perspective, as well as his illustrative and artistic powers, to attribute to him such an obvious failure to make good his intentions, when he had so much experience in the construction of asymmetric stereometric bodies'⁸. What the artist seems to have wanted to do was to make the solid difficult to identify, in which he succeeded all too well. In order to confirm Panofsky's conclusion, a truncated model of the edges of a cube was

Fig. 1 (right) *Melencolia I*, copperplate engraving (1514) by Albrecht Dürer (239 x 168 mm), National Gallery of Art, Washington DC. (Permission to reproduce granted by letter, October 1980.) **Fig. 2** (below, upper diagram) Model of the edges of a cube truncated so that the edge of the triangular face bears a ratio to the intact diagonal of the principal upper face similar to that in the figure in *Melencolia I*. (Paul Framer photo.) **Fig. 3** (below, lower diagram) Model of the edges of a truncated acute rhombohedron, apical angle of rhombs 72°, intact diagonal of principal upper face equal to that of the cube above (Fig. 2) and edge of triangular face in the same ratio to it. (Paul Framer photo.)

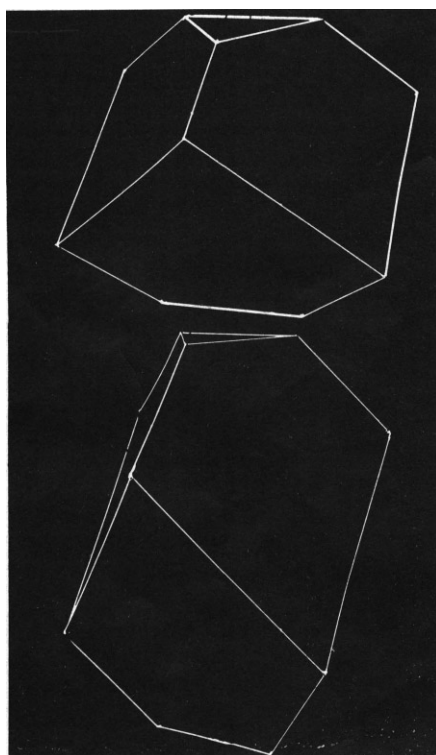


IMAGE
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REASONS

photographed from a point of view approximating that in the engraving. The intact summit at the centre of the image and the angle between the upper two cube faces enclosed by the visible edges differ conspicuously from the *Melencolia* solid (Fig. 2).

Max Steck, thinking it might be a modified octahedron, enlisted the gemologist Paul Grodzinski to construct a model of the solid. Starting with the plane figure of eight identical equilateral triangles from which the octahedron develops, Grodzinski reduced the size of two opposing faces and redrew the others as identical five-sided polygons. When this plane figure was cut out and folded, the resulting solid resembled the *Melencolia* body but was too slender. A cube he constructed with opposed summits truncated by equilateral triangles was too oblique. For his third trial, he drew five-sided plane figures with apical angles of 72° ('as the angle of 72° is connected with the polygon') and obtained a solid more closely resembling the artist's image. The key to creating the solid was 'the point angle used by Dürer', he reported. 'This is offered as a solution to the question of what the geometric body in Dürer's *Melencolia* represents'⁹. When an edge model with these apical angles (and opposed diagonals equal to those of the cube above) was constructed and photographed from the same distance, the edges and internal angles of the resulting image approximated those in the engraving (fig. 3).

Although Dürer's sketch for the figure did not detail the steps by which he constructed it, for his treatise on measurement he drew the plane figure from which develops a tetrahedron truncated by an opposing one of different size¹⁰. This shows that he had apprehended one of the geometrical properties of the octahedron, in which two identical tetrahedra are symmetrically opposed. Another diagram shows that he realized that the summits of a cube can be truncated equally by the faces of an octahedron. If only two summits are altered in any perfect solid, its other axes of symmetry are lost and its spatial logic is destroyed¹¹.

Attribution of meanings to the manifestations of culture is a subjective process. Panofsky has persuasively argued that the history of art is a humanistic discipline because that is what it seeks to do¹². When the *Melencolia* body is regarded as a geometrical problem, without reference to what it might mean but simply what solid it represents, we may specify that the solution would be a figure that could be truncated by equilateral triangles without losing its spatial logic. This could only be a trigonal solid exhibiting threefold rotational symmetry. The geometrical figure that meets this condition is the rhombohedron, which can be truncated on its principal axis without losing its rotational symmetry. Since two visible summits are oblique, the terminations removed would



100 years ago

THE THIRST FOR SCIENTIFIC RENOWN

FEW students of science can fail to feel at times appalled by the ever-increasing flood of literature devoted to science and the difficulty of keeping abreast of it even in one special and comparatively limited branch of inquiry. Were merely the old societies and long established journals to continue to supply their contributions, these, as they arrive from all parts of the country, and from all quarters of the globe, would be more than enough to tax the energy of even the most ardent enthusiast. But new societies, new journals, new independent works start up at every turn, till one feels inclined to abandon in despair the attempt to keep pace with the advance of science in more than one limited department.

be less than right angles. The figure may thus be identified as a truncated acute rhombohedron.

A crystallographer, Charles Bunn¹³, wrote that the *Melencolia* body was 'like an enormous crystal . . . challenging the human onlooker by the precision and symmetry of its form' (without suggesting its identity). Dürer seems to have had some intuition of the symmetry properties of the body since he invited comparison of the polyhedron to the sphere below it in the composition. The sphere exhibits perfect rotatory symmetry while the sleeping dog, even though it curls in a circle, has none. Not until almost three centuries later would naturalists observe that the mineral calcite, when it takes the form of the acute rhombohedron in crystals familiarly known as dog-tooth spar, sometimes occurs in truncated examples without loss of symmetry¹⁴. Some of these are less acute than that in Fig. 3¹⁵. The failure of art historians to establish the geometric identity of the figure has allowed them to overlook one of the dramatic qualities of the work, that the artist knew the answer to the puzzle that perplexes the heroine of the engraving¹⁶. An historian of chemistry and alchemy suggested that it might be the 'image of the philosopher's stone or . . . of that so-called 'Stone of Saturn' ' in some ritual¹⁷. It would be more fitting to credit Albrecht Dürer with anticipatory intuitions about the science of descriptive geometry established long after his death. What appears in this remarkable work of art is that by 1514 magic had lost its grip on the educated mind¹⁸. □

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One of the most striking and dispiriting features of this rapidly growing literature is the poverty or worthlessness of a very large part of it. The really earnest student who honestly tries to keep himself acquainted with what is being done, in at least his own branch of science, acquires by degrees a knack of distinguishing, as it were by instinct, the papers that he ought to read from those which have no claim on his attention. But how often may he be heard asking if no means can be devised for preventing the current of scientific literature from becoming swollen and turbid by the constant inpouring of what he can call by no better name than rubbish!

Some sciences seem to be specially exposed to inundation of this kind. Geology lies exposed to it in an unusual degree. Popular in its subject, and capable of ready apprehension as to its general principles, this department of science allures the outsider into its precincts, where he too frequently soon arrives at the belief that to have read a geological book or two is to become a geologist. This belief would be harmless enough, did it not speedily bear fruit in 'papers' communicated to scientific journals, and stamped with all the enthusiasm and crudity of a beginner.

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Early man

A new chronology for the Mousterian

from Robin Dennell

A QUIET revolution in studies of the Upper Pleistocene has resulted in a doubling of the estimated duration of the Mousterian stone tool industries of the last glaciation and, as a consequence, of the Neanderthal populations responsible for them. The change is important in that it revives old controversies and presents a new paradox.

The redating suggests that the period between the last classic Neanderthal and the emergence of modern man may, after all, have been long enough for *neanderthalensis* to have evolved directly into *sapiens* in Europe and calls into question the now more widely-held view that the Neanderthals disappeared after the immigration of *sapiens* into Europe from elsewhere (see *News and Views* 287, 271; 1980). At the same time the redating suggests that Mousterian industries remained virtually unchanged over very long spans of time — in marked contrast to the speed with which modern man's lithic stone tool industries advanced during the Upper Palaeolithic — and suggests fundamental differences in Neanderthal and *sapiens* societies that make it more difficult to see how one could have evolved into the other.

According to the traditional chronology, the Mousterian of the last glaciation was confined to a period of some 30 kyr that lasted from the end of the last interglacial to its replacement by Upper Palaeolithic industries some 35 kyr ago. The lower limit of the Mousterian hinged on the dating of the end of the last (Eemian, Ipswichian, Riss-Würm) interglacial. Estimates that this ended around 65 kyr ago were derived from ^{14}C dates on organic matter preserved during the earliest mild episodes (for example, Brørup) of the last (Weichsel, Devensian, Würm) glaciation that was assumed to have directly followed it. The approach had two major limitations. First, it did not provide any

guarantee of stratigraphical continuity between the end of the last interglacial and the beginning of the last glaciation; second, the dates obtained lay at the very limit of reliable ^{14}C dating techniques and were thus particularly prone to error. Various studies — particularly of the sediments and associated pollen and fauna of caves in south-west France¹ — divided the part of the last glaciation that was associated with Mousterian industries into two: an initial mild part, in which the red deer was the commonest ungulate; and a later reindeer-dominated part. Whilst this relative sequence still holds good, its dating within the traditional framework is now highly suspect.

The new chronology has resulted largely from studies of deep-sea cores and raised coral beaches. In 1969, Shackleton² showed that oxygen isotope stage 5, previously assigned in its entirety to the last interglacial, has five sub-stages, of which only the earliest, 5e, seemed to have been interglacial. Later, Shackleton and Matthews³ demonstrated that gastropods from the Barbados III terrace in the Caribbean had a similar isotopic composition both to modern (interglacial) ones and to foraminifera from those parts of deep-sea cores that could be assigned to stage 5e. This terrace was dated by Th/U to around 125 kyr BP. Further confirmation has since come from two cores that were analysed for their pollen and foraminiferal content, one taken off the coast of Norway⁴ and the other from offshore British Columbia⁵; in both cases, only stage 5e had a truly interglacial pollen spectrum. Estimates that this interglacial lasted less than 10 kyr and was terminated by 115 kyr BP has come from a variety of sources, including studies of varved deposits and clusters of Th/U dates on fossil corals on islands such as Hawaii⁶.

What was not suspected until recently was that the last interglacial was not

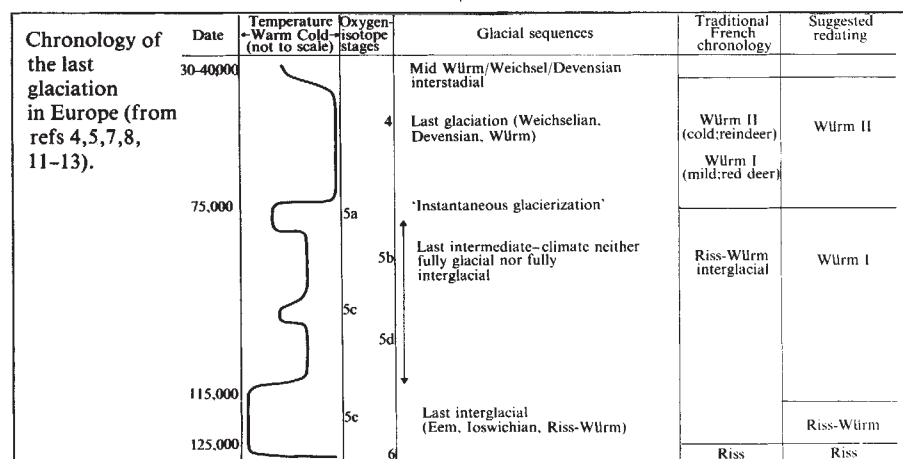
followed immediately by a glaciation, as implicitly assumed in the traditional chronology. Instead, details are now emerging of a 40 kyr interval, during which 'the world was in neither a full glacial nor a full interglacial mode'⁷.

According to Woillard's⁸ study of the finely calibrated pollen profile from Grande Pile in northern France, the transition from a temperate to a boreal forest was accomplished in about 150 ± 50 yr. Studies of fossil beaches on islands that have experienced considerable tectonic uplift in the last 100 kyr indicate that sea levels dropped to 20 and 70 m below present level between 115 and 75 kyr ago (compared with over 100 m during the glacial maximum of 18 kyr BP; see, for example, ref. 9). In the North Atlantic, sub-tropical and intermediate water advanced twice in this interval, only to be displaced by polar waters¹⁰; on land, evidence from Grande Pile shows two post-Eemian but pre-Weichsel warm phases, called St Germain I and II, that punctuated the cold Melissey I and II phases.

It was only after 75 kyr ago that fully glacial conditions prevailed. In the North Atlantic, polar water extended far further south than today, and the amount and area of ice-rafted debris increased dramatically¹¹. At Grande Pile, a rapid replacement of trees by grassland is indicated by around 70 kyr ago¹². The speed with which this cooling was effected has led some authors (for example, Andrews and Barry¹³) to summarize it as one of 'instantaneous glacierization' that resulted in the rapid expansion of land-based ice sheets over much of northern America and Europe.

The most obvious effect of this new chronology is that the south-west French sequence for the Mousterian of the last glaciation should probably be stretched to fit a period twice as long as previously believed (see the figure). This redating has two consequences that, as mentioned earlier, result in a paradox. On the one hand, the stability — and even stagnation — of Mousterian tool-making traditions becomes even more striking than before. Thus if one accepts that Mousterian industries remained virtually unchanged over 70 kyr (but see ref. 14), Neanderthal

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society would seem to have been fundamentally different from that of modern man after 30–35 kyr ago. On the other hand, the rates of Neanderthal evolution between the end of the last interglacial and 35 kyr ago may have to be halved. The interval between the latest classical Neanderthals and the earliest *Homo sapiens sapiens* in Europe may have been sufficiently long for the former to have evolved into the latter. Unfortunately, most Neanderthal remains are still very poorly dated, and will remain so until the incoming generation of ^{14}C techniques provide reliable age determinations back to 100 kyr ago¹⁵.

Nevertheless, many of these finds may turn out to be much older than previously thought, and the gaps between them much longer. Indeed, the important Mousterian site of Tata in Hungary has recently been redated¹⁶ from 55 to 110 kyr BP. Whether the archaeological evidence that Neanderthal behaviour was fundamentally different in its conservatism from that of *Homo sapiens sapiens* can be reconciled with the possibility that Neanderthals evolved in an almost leisurely fashion into modern man remains to be seen. □

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Molecular technology

Designing proteins and peptides

from Carl Pabo

ADVANCES in DNA synthesis make it possible to change specific amino acid residues in a protein (by directed mutagenesis) or to create new proteins by synthesizing the required gene. These advances have led to recent discussions about directed modification of protein structure and function¹ and to speculation about designing novel proteins that could be used as 'molecular machinery' for a futuristic microtechnology². Even though we are unable to predict reliably three-dimensional structures from a knowledge of amino acid sequences, there are already examples of the modification of the sequence and structure of peptides in a rational manner. A corresponding ability to modify existing proteins or to design new proteins would have very important theoretical and practical implications.

Several reports show that the rational modification of peptides, based on secondary structure predictions and model-building, is now feasible. Synthetic peptides have been made which retain biological function and appropriate secondary structure, even though they have a very limited sequence homology with the natural peptide³ or are much smaller⁴. For example, several studies with hormones have indicated that it is possible to stabilize a β -turn by cyclization of the molecule, either by introducing a disulphide bond⁵ or by designing a cyclic peptide⁴. The approach has yielded some peptides which are more active and longer-lasting than the natural peptides. For example, the replacement of nine residues of somatostatin with a single proline residue⁴ gives a cyclic hexapeptide which is significantly more active than somatostatin itself.

Amphiphilic synthetic helices provide another example of rational peptide design based on considerations of secondary structure. Such helices mimic the biological action of apolipoprotein⁶ or the action of melittin³, even though these helices have very limited sequence homology with the natural protein or peptide. The analogue of

melittin differs from the normal sequence at 15 of the first 20 residues (although 6 of the changes are rather conservative) and is homologous only in the C-terminal hexapeptide region (residues 21–26). In spite of these changes, circular dichroism studies suggest that this synthetic peptide forms an α -helix, and the peptide is more active than melittin in lysing erythrocytes.

At least one ambitious attempt to design a larger peptide, that would contain a mixture of secondary structures, has been reported⁷. A 34-residue polypeptide was designed so that it might form two antiparallel β -strands and an α -helix. The expected arrangement of the backbone and the amino acid sequence were chosen so that the peptide monomer might bind to the anticodon of tRNA^{Phe}. The peptide was synthesized, and it appeared to have some affinity for RNA, since a peptide dimer (covalently linked by disulphide bonds) had some RNase activity on a poly(C) substrate. Unfortunately, no detailed structural information was available, so it was not possible to evaluate the accuracy of the structural prediction.

What is the prognosis for modifying and designing proteins? Ulmer has recently discussed the prospects for directed modification of protein structure and function¹. He sidesteps the protein-folding problem by restricting the study to cases where the three-dimensional structure of the protein has been determined by X-ray crystallography and then considers the prospects for planning sequence changes that would give changes in the activity or physical properties of the protein.

Drexler² speculates that it should be possible to design novel proteins and that such proteins could provide a 'general capability for molecular manipulation'. He points out that it may not be necessary to solve the protein-folding problem before we are able to design proteins. The protein designer can choose from an extremely large number of possible amino acid sequences. (A 100 residue protein could

have any one of 20^{100} sequences.) Even if only a vanishingly small percentage of these possible sequences give a predictable fold, it might be possible to design proteins, since the engineer could choose to work with this small subset of sequences.

How might one find any rare sequences which would give a predictable fold? Drexler does not discuss this point, but one might proceed, as Gutte *et al.*⁷ seem to have done, by designing a fold for the protein backbone before picking an amino acid sequence. Thus, rather than starting with an amino acid sequence and then predicting the conformation of the folded polypeptide, one starts with a conformation of the backbone and then picks an amino acid sequence that should stabilize it. This 'inverted' approach might be useful, because we know more about the characteristics of folded proteins than we do about the process of folding. The approach should allow all of the principles gleaned from X-ray crystallography and structural analysis of proteins to be used directly. For example, knowledge of the secondary structure of the 'prefolded backbone' could be used in the same way that it has been used in the design of peptides. If a section of the backbone has been assigned to an α -helical conformation, one would only add residues with high helical potential. However, the real advantage of the 'inverted' approach, and this is a feature which Gutte *et al.* do not mention, should come when tertiary interactions are considered. 'Inversion' eliminates the problem of predicting long-range interactions, since residues which will interact in the tertiary or quaternary structure are already close in space when they are added to the pre-folded backbone. One knows which residues will be close in three-dimensional space, and one should be able to pick residues which will have favourable interactions with their neighbours.

It may be difficult to design proteins which carry out particular functions, but the use of a pre-folded backbone should also be helpful at this stage. In fact, the inverted approach may simplify protein design even after the folding problem is solved. Since the final folded structure of a protein must always provide the basis for predicting or modifying the function, it would seem most efficient to start with the backbone in a particular configuration and then choose a sequence which gives the desired arrangement of reactive residues. □

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Nature of the Kirkwood gaps in the asteroid belt

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The distributions of orbital eccentricities e and inclinations I near the jovian resonances in the asteroid belt show that the observed Kirkwood gaps in the distribution of the semimajor axes were formed after the asteroids had dispersed from the near-coplanar disk in which they accreted.

THE gaps in the distribution of the asteroidal semimajor axes at the locations of the jovian resonances were discovered by Kirkwood¹ in 1867 at a time when only 91 asteroidal orbits were known. Since then more than 2,000 reliable orbits have been catalogued, and many theories have been proposed to account for the formation of the gaps², but a consensus of opinion has yet to be achieved. Kirkwood gap formation remains one of the major outstanding problems of planetary dynamics.

The various theories of gap formation have been divided by Greenberg and Scholl² into four classes: (1) the cosmogonic hypothesis (the gaps represent regions where asteroids failed to form during the early history of the Solar System); (2) the statistical hypothesis (the proposal that asteroids are librating about the gaps and therefore are only rarely seen crossing the exact resonances); (3) the collisional hypothesis (the gravitational effects of Jupiter increase collision rates within the gaps causing a wearing down or removal of material); (4) the gravitational hypothesis (the gaps are caused by purely gravitational interactions with Jupiter). For the present purposes, we need to divide the latter class into first those theories in which Jupiter acts on asteroids moving in a near-coplanar disk (these theories may also belong in class (1)) and second those theories in which Jupiter acts on the present distribution of orbital elements.

No doubt all of these hypotheses could account for gap formation in some ideal system, but to decide whether they account for the present gaps in the asteroid belt we must appeal to observation. Pioneers of asteroidal dynamics^{3,4}, realizing that the gaps may not simply be regions of low number density, did attempt to define further observed characteristics of the gaps. By finding the mean eccentricity of the 10 asteroids on either side of each gap, Hirayama⁴ confirmed the statement of Brown³ that the eccentricities of asteroids near the Kirkwood gaps were lower than the mean eccentricity of all the asteroids. However, Hirayama failed to attach any statistical significance to his result. Nevertheless, the findings of Brown and Hirayama have been unjustifiably overlooked, leading theorists, unconstrained by observations, to entertain too wide a variety of hypotheses.

More recently Zellner and Bowell⁵ suggested that there is a tendency for the large mass asteroids to avoid the gaps while Chapman⁶ claimed that the distribution of asteroidal types is to some extent determined by the location of the Kirkwood gaps. We⁷ applied statistical techniques to the orbital elements contained in the TRIAD (Tucson Revised Index of Asteroid Data) file in an attempt to define the properties of the Kirkwood gaps and thereby provide an observational framework for investigating theories of their origin. By examining a data set free of observational selection effects we concluded that there was a significant tendency for asteroids near the Kirkwood gaps to have not only lower eccentricities but also lower inclinations. We demonstrated the need to examine a data set free of observational selection effects by showing that the 'mass effect' observed by Zellner and Bowell⁵ was the result of an observed

excess of small mass objects with low inclinations. This excess is a consequence of the tendency for asteroids to be discovered in the plane of the ecliptic⁸. Here we strengthen our arguments by investigating the properties of individual Kirkwood gaps and by relating the observed distributions of orbital elements to the results of standard resonance theory. We also investigate the large-scale distribution of eccentricities.

Resonance

The magnitudes and the phases of the perturbations of an asteroid's near-keplerian orbit are determined⁹ by the jovian disturbing potential U . This can be expanded as an infinite summation of terms, a Fourier series, with the general form^{10,11}

$$U = \sum S \cos \phi \quad (1)$$

where, to lowest order in eccentricity and inclination,

$$S = (Gm'/a')(-1)^q f(\alpha) e^m (\sin \frac{1}{2} I)^{q-m} \quad (2)$$

and the argument ϕ has the form

$$\phi = (p+q)\lambda' - p\lambda - m\tilde{\omega} + (m-q)\Omega \quad (3)$$

where m , p and q are integers, a is the semimajor axis, e the eccentricity, I the inclination, λ the mean longitude, $\tilde{\omega}$ the argument of pericentre, Ω the longitude of ascending node, $\alpha = a/a'$ and $f(\alpha)$ is a function that has to be evaluated for each resonance. The orbital elements of Jupiter are denoted by primed quantities and its gravitational mass by Gm' .

An asteroid is trapped in a resonance if some argument ϕ , the resonant argument, librates rather than circulates¹². The integer q , the order of the resonance, largely determines the strength S of the resonance: as $|q|$ increases in integral steps, $|S|$ decreases in steps of $\sim e$ or $\sim \sin \frac{1}{2} I$. The locations and the strengths of all resonances of order $q \leq 11$ between the 3:1 resonance at 2.50 AU and the 4:3 resonance at 4.29 AU are shown in Fig. 1.

If the resonant argument ϕ librates, then the asteroidal orbit is near-periodic and the orbital elements oscillate about their mean values with a common period. If the amplitude of libration of ϕ is a maximum, then the range through which the semimajor axis oscillates is also a maximum and we refer to this range as the libration width W . If $\tilde{\omega}$ and Ω are negligible compared with $(p+q)n' - pn$, then, using Lagrange's planetary equations⁹, we

Table 1 Strengths of lowest order terms

Resonance	a (AU)	p	q	m	$q-m$	$f(\alpha)$
2:1	3.2776	1	1	1	0	1.1905
3:1	2.5012	1	2	2	0	0.5988
		1	2	0	2	0.3312
5:2	2.8245	2	3	3	0	1.1329
		2	3	1	2	1.2888
13:6	3.1072	6	7	7	0	21.2753
		6	7	5	2	80.1896
		6	7	3	4	70.7032
		6	7	1	6	12.3861

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can show that the equation of motion of ϕ is

$$\ddot{\phi} = \begin{Bmatrix} - \\ + \end{Bmatrix} \frac{3p^2|S| \sin \phi}{a^2} \quad (4)$$

where the upper half of the brackets refers to the case $S < 0$ and the lower half to the case $S > 0$. By finding the energy integral for maximum libration and comparing it with the time derivative of equation (3), we find the maximum deviation of mean motion from the value n_0 given by $(p+q)n' = pn_0$, to be

$$\delta n = \pm \left(\frac{12|S|}{a^2} \right)^{1/2} \quad (5)$$

Hence, from Kepler's third law, we have for the libration width (cf. ref. 13)

$$W = 8 \left[\frac{|S|}{3n^2 a^2} \right]^{1/2} a \quad (6)$$

If $\ddot{\omega}$ and $\ddot{\Omega}$ are not negligible compared with $(p+q)n' - pn$, then equation (5) must be modified. We find that the extreme values of δn are given by

$$\delta n = -\frac{g|S|}{3pna^2} \pm \left[\frac{12|S|}{a^2} \right]^{1/2} \left[1 + \frac{g^2|S|}{27p^2n^2a^2} \right]^{1/2} \quad (7)$$

where

$$g = \frac{m^2}{e^2} - \frac{m(m-q)}{2} + \frac{(m-q)^2}{4 \sin^2 \frac{1}{2} I} \quad (8)$$

The first term on the right of equation (7) represents a shift in the location of exact resonance which must occur when $\langle \ddot{\omega} \rangle$ or $\langle \ddot{\Omega} \rangle$ are not zero. The second term is the maximum oscillation in n that can occur and thus this term gives the modified libration width. For all resonances, except the 2:1 and all other first-order resonances, the shift in the location of exact resonance and the change in W are small and equation (6) is adequate for our purposes.

In this analysis we have assumed that on integrating $\ddot{\phi}$ in equation (4), S can be treated as a constant. This is equivalent to assuming that the forced eccentricities and inclinations are negligible compared with their free values—a good approximation for most asteroids, particularly if the order q of the resonance is high. With this approximation it can now be stated that unless an asteroid has a semimajor axis in the range bounded by W , resonance is impossible and the associated argument ϕ must circulate. We now discuss the relation of W to the widths of the observed gaps.

Gap widths

Our aim is to relate the results of resonance theory to the observed asteroidal distributions in $a-e$ space and $a-\sin \frac{1}{2} I$ space. However, since the resonance strength S is a complicated function of both e and I , to progress we must make several approximations.

Since $\ddot{\omega}$ and $\ddot{\Omega} \ll n$, $n'/n \approx p/(p+q)$ and the approximate location of a resonance is determined by p and q alone. For each pair of p and q , there are an infinite number of possible resonances, but most of these are very weak. Some examples of these possible resonances, and their associated values of $f(\alpha)$, are given in Table 1. For asteroids in the main belt, we observe that $\langle e \rangle / \langle \sin \frac{1}{2} I \rangle = 1.32$, hence there is a tendency, particularly if q is high, for the strongest resonances to be those for which $q-m$ is low, if not zero. For eccentricity-, or e -type, resonance¹¹, $q-m$ is zero and the leading term in the expansion of U is independent of I (see equation (2)); higher-order terms do depend on I , but these are usually small unless e or I is high. If q is even, then an inclination-, or I -type, resonance¹¹ is possible for which m is zero and the leading term in the expansion of U is independent of e . Accordingly, it is reasonable to compare the distribution of asteroids in $a-e$ space with the libration width associated with the leading term in the expansion of U for e -type resonance ($q-m=0$), and, for even order resonances, to compare the distribution of asteroids in $a-\sin \frac{1}{2} I$

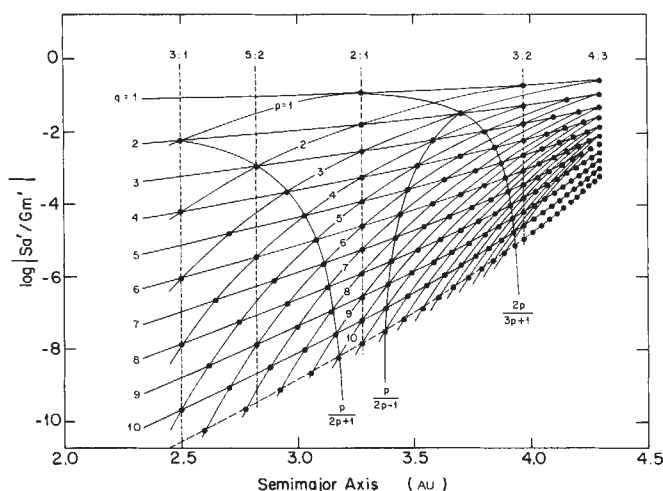


Fig. 1 Resonances occur when $n'/n = p/(p+q)$, where n'/n is the ratio of the jovian and asteroidal mean motions and p and q are integers. The semimajor axis corresponding to a particular resonant n can be located by finding the point of intersection of the appropriate p and q curves, for example, the 8:3 ($=p+q:p$) resonance is located at the intersection of the $p=3$ and the $q=5$ curves at 2.70 AU. The ordinate gives the strength of the resonance, that is, the leading eccentricity term in the expansion of the disturbing function. All resonances of order $q \leq 11$ between the 3:1 resonance at 2.50 AU and the 4:3 resonance at 4.29 AU, and their strengths S in dimensionless units for the case $e=0.1$ are shown. The spacings of the resonances in the $p/(2p+1)$, $p/(2p-1)$ and $2p/(3p+1)$ series decrease as p increases and we find that these resonances overlap at modest values of e (≤ 0.3).

space with the libration width associated with the leading term in the expansion of U for I -type resonance ($m=0$).

The distribution of asteroids in $a-e$ space in the vicinity of the 3:1 resonance at 2.50 AU is shown in Fig. 2a. Also shown are the bounds of the libration region in that plane. The evidence of this figure alone is sufficient to eliminate two of the hypotheses for gap formation. For the statistical hypothesis to be tenable, most of the asteroids near the gap must be librators. However, we observe that that region in $a-e$ space where libration is possible is almost devoid of asteroids, thus the observed Kirkwood gap cannot possibly be a statistical phenomenon: this supports the conclusions of several other authors¹⁴⁻¹⁷. We note that some of the few asteroids that remain in the libration region have exceptionally high eccentricities, high enough indeed to be Mars-crossers. This supports the suggestion of Zimmerman and Wetherill¹⁸ that the asteroids that are presently in gaps will have their eccentricities pumped up by jovian perturbations to values high enough for them to be eventually removed by close planetary encounters. Impressive progress with the dynamics of this process, which may not only account for Kirkwood gap formation, but may also account for the delivery of meteorites from the asteroid belt to the Earth, has recently been made by Wisdom¹⁷.

The distributions of the orbital elements outside the libration regions are also of interest. Figure 2b shows the variation of mean number density, mean proper eccentricity and mean proper inclination for non-family asteroids near the 3:1 resonance (non-family asteroids alone are used in these plots to avoid those problems associated with the non-randomness of the distributions of the orbital elements of family members⁷). Figure 2b is clear evidence that eccentricities and inclinations tend to be low near a strong resonance⁷. This is partly to be expected from the shapes of the libration regions in the $a-e$ and the $a-\sin \frac{1}{2} I$ planes. However, it is equally clear from Fig. 2b that the widths of the libration regions are much less than the widths of the observed gaps. The curves shown in Fig. 2b were obtained using a running box¹⁹ with a box width of 20 asteroids. We have confirmed that changing, and in particular reducing, the number of asteroids in the box has little effect on the observed widths of the gaps: the disparities between these widths and

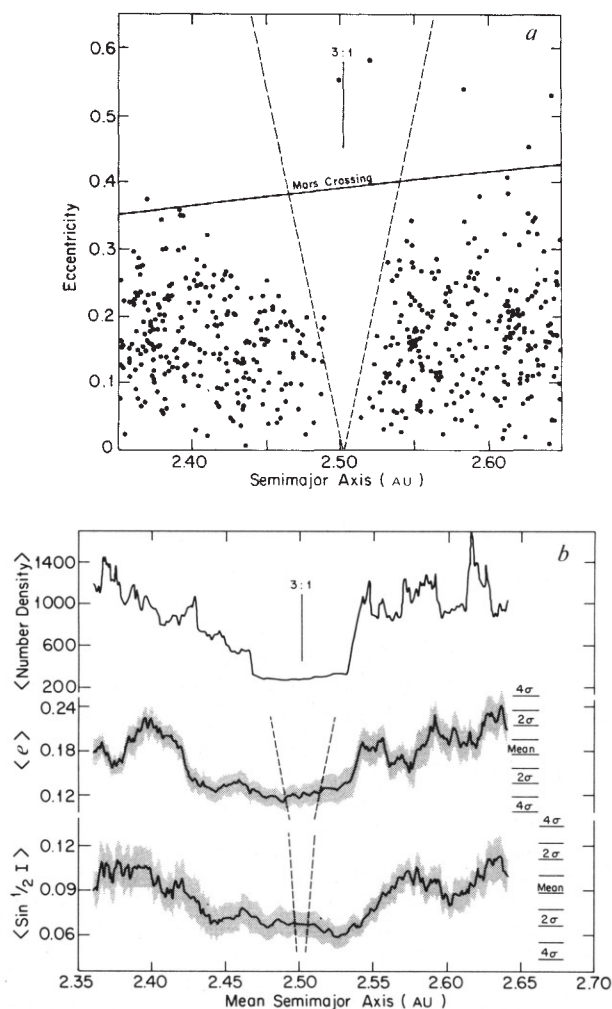


Fig. 2 *a*, Distribution of the osculating eccentricities of all asteroids with $2.35 < a < 2.65$ AU listed in the TRIAD file. The dashed lines represent the libration width associated with the leading eccentricity term in the expansion of the disturbing function. Asteroids with high eccentricities cross the orbit of Mars and may be removed by close planetary encounters. *b*, Plots of mean number density (asteroids/AU), mean proper eccentricity e and mean $\sin \frac{1}{2} I$, where I is the proper inclination, for all non-family asteroids with $2.35 < a < 2.65$ AU listed in the TRIAD file, obtained by applying a running box method¹⁹ to the 242 asteroids in the chosen population which were sorted in order of increasing semimajor axis a . The running box, and hence each sample, contained 20 asteroids and was stepped through the population one asteroid at a time. The σ levels on the right refer to the distribution of the means of the samples and were estimated from the variance of the population as a whole¹⁹. The hatched areas are the envelopes of the 1σ error bars of the samples which were estimated from each sample variance. The dashed lines represent the libration widths associated with the leading eccentricity term ($\langle e \rangle$ plot) and the leading inclination term ($\langle \sin \frac{1}{2} I \rangle$ plot) in the expansion of the disturbing function.

the widths of the libration regions are real and are not artefacts of our statistical method.

The most important conclusion to be drawn from Fig. 2*a, b*, is that the resonant locations in the asteroid belt can be detected in the *present* distributions of eccentricities and inclinations. It follows that the Kirkwood gaps that we now observe must have been formed after the asteroids had dispersed from the near-coplanar disk in which they accreted. This observation eliminates the cosmogenic hypothesis of gap formation. We do not deny that gaps may have been formed in the accretion disk, but only contend that these would have no relation to the observed structure in the present a - e space of the asteroids which must have been formed later.

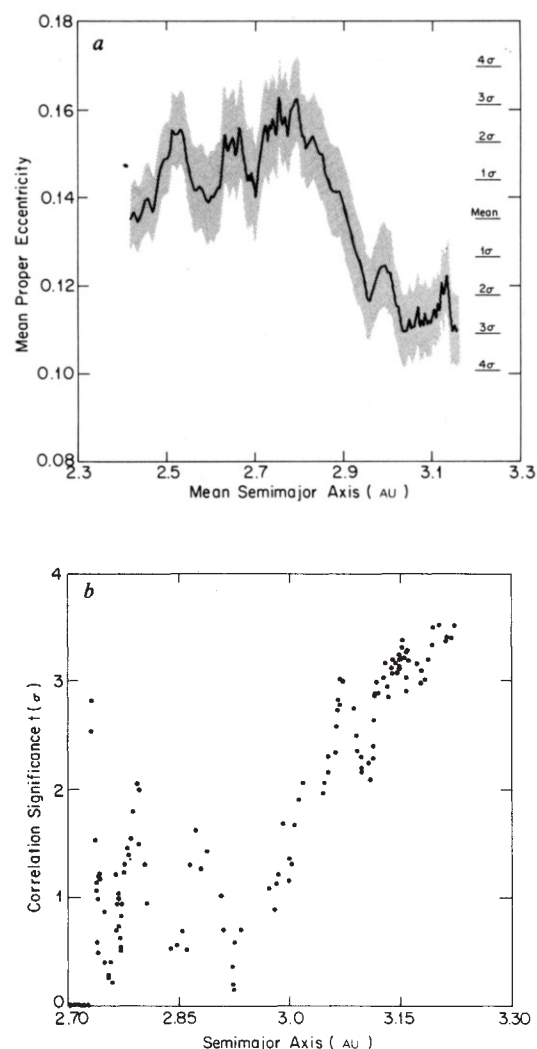


Fig. 3 *a*, Variation of mean proper eccentricity e with mean proper semimajor axis a for the 236 asteroids in our bias-free set. The running box contains 40 asteroids. *b*, Variation with increasing a of the statistical significance t (in standard deviations σ) of the linear correlation between e and a for the asteroids in our bias-free set with $a > 2.7$ AU.

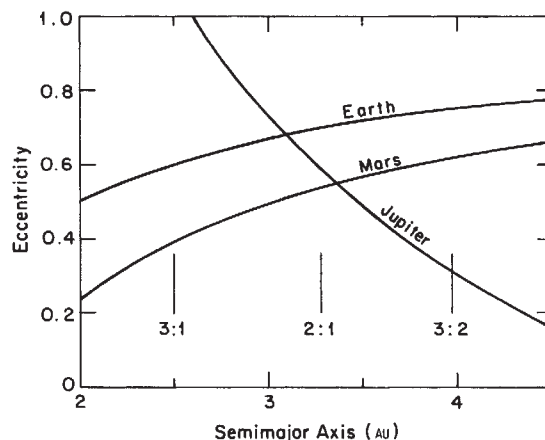


Fig. 4 Orbital eccentricities needed to cross the mean orbits of Earth, Mars and Jupiter.

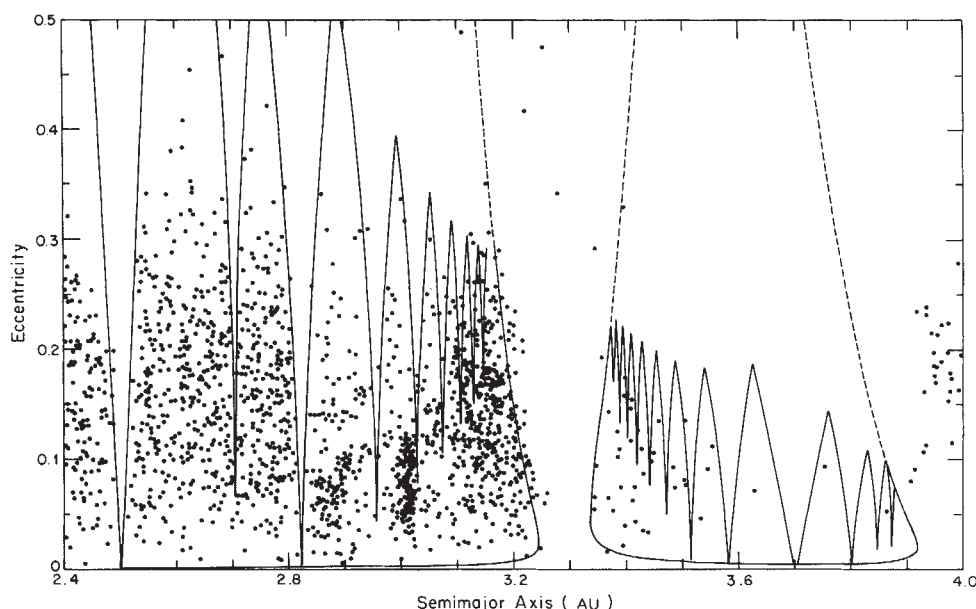


Fig. 5 Distribution of the osculating eccentricities of all asteroids with $2.4 < a < 4.0$ AU listed in the TRIAD file. The solid lines represent the libration width associated with the leading eccentricity term in the expansion of the disturbing function. All resonances in the $p/(2p+1)$, $p/(2p-1)$ and $3p/(3p+1)$ series, and the 8:3 resonance at 2.70 AU, are shown. Resonance overlap occurs where the solid lines meet. In the region of resonance overlap, the libration widths of the 2:1 resonance of 3.3 AU and the 3:2 resonance at 4.0 AU are represented by dashed lines.

Distribution of eccentricities

The strongest trends that exist in the TRIAD file, for example, the decrease of mean absolute magnitude with increasing semimajor axis, are merely the results of observational selection. However, although these selection effects are easy to recognize and understand, they are difficult to allow for. We consider⁷ that the real structure of the asteroid belt is best revealed by that group of asteroids for which observational selection effects can be discounted and for which bias-corrections are unnecessary: these asteroids are simply the brightest. As in our previous paper⁷, we consider only those asteroids of absolute magnitude $B(1, 0) < 9.68$ and only the largest member of each family is retained in the data set. In the range $2.2 < a < 3.27$ AU there are 236 asteroids in this category: these asteroids constitute our bias-free set.

The variation of mean proper eccentricity with mean proper semimajor axis for the asteroids in our bias-free set is shown in Fig. 3a. The curve was obtained using the running box method¹⁹ with a box width of 40 asteroids, large enough to smooth out any variations due to the individual resonances. $\langle e \rangle$ increases from 0.135 at $\langle a \rangle = 2.40$ AU to a maximum of 0.160 at $\langle a \rangle = 2.80$ AU. This modest trend could be due to gravitational sweeping of asteroids in high eccentricity orbits by Mars²⁰ and by Earth (see Fig. 4). But gravitational sweeping cannot account for the marked decrease in $\langle e \rangle$ that we observe beyond $\langle a \rangle = 2.80$ AU. Gravitational sweeping by Jupiter would affect only those asteroids in highly eccentric orbits. The variation of the significance t (Student's statistic²¹ in standard deviations σ) of the linear correlation coefficient with increasing semimajor axis for asteroids in the range $2.7 < a < 3.3$ AU is shown in Fig. 3b. We consider that this tendency for the mean eccentricity to decrease with increasing semimajor axis, which is significant at $>3\sigma$ level, is due to resonance overlap and is further evidence of post-formative orbital evolution in the asteroid belt.

Resonance overlap

The distribution of asteroids in $a-e$ space in the range $2.4 < a < 4.0$ AU and the libration widths of the strongest resonances in that range are shown in Fig. 5. The strongest resonances are those in the series $n'/n = p/(2p+1)$, $p/(2p-1)$ and $2p/(3p+1)$ (see Fig. 1). The only other strong resonance, which is not in those series, but which is shown in Fig. 5, is the 8:3 resonance at $a = 2.70$ AU.

It is evident from Fig. 1 that the strengths of the resonances in the $p/(2p+1)$, $p/(2p-1)$ and $2p/(3p+1)$ series decrease as p increases. However, it is equally evident that the spacings of the resonances also decrease. We have found that this decrease

is so marked that the libration regions overlap at modest values of e , and that in the case of the important $p/(2p+1)$ series, which lies between the 3:1 resonance at 2.50 AU and the 2:1 resonance at 3.27 AU, overlap occurs at progressively smaller values of e as p , and a , increase. The reality of this phenomenon, and the efficacy of the approximations that we have used in our calculations of the libration widths, have been confirmed by an analysis based on a nineteenth-order expansion of U .

In Fig. 5, we see that where the libration regions overlap, the Kirkwood gaps are no longer localized and large regions of $a-e$ space are devoid of asteroids. The envelope of those points where adjacent gaps first overlap places an upper bound on the asteroidal eccentricities and the shape of this envelope would appear to account for the trend in the mean eccentricities that we have shown to exist.

Figure 5 also confirms that the 3:1 resonance is typical, in that those regions bounded by the libration widths are zones of exclusion. The 2:1 resonance at 3.3 AU is particularly striking. We note that, as in the case of the 3:1 resonance, those few asteroids that remain in the centre of the libration region tend to have eccentricities much higher than the mean eccentricity of their immediate neighbours. We have also found, using the family definitions of Williams²², that the ratio of the family to the non-family asteroidal number densities decreases as the bounds of the libration region are approached and that most asteroids in the libration regions are non-family asteroids. This could be due to the fact that no allowance has been made for resonant perturbations in the derivation of the asteroidal proper elements and, consequently, that some family members have not been recognized as such. This possibility, which has a bearing on the existence of Kirkwood gaps in Hirayama families⁷, needs further investigation. Of, perhaps, more interest is the possibility that resonant perturbations have acted to disperse the proper elements of family members, in which case the observed excess of non-family members would be further evidence of post-formative orbital evolution. Orbital evolution may result in the continuous injection of asteroids, of all sizes, into the inner edge of the 2:1 libration regions, from which they are eventually removed by that process which is responsible for the clearing of the Kirkwood gaps. This could account for those asteroids which lie just inside the inner edge of the 2:1 libration region.

Conclusions

We have demonstrated that the Kirkwood gaps are not merely regions of low asteroidal number density, but are regions in $a-e-\sin \frac{1}{2}I$ space where libration of some argument ϕ is possible.

We have argued that neither the statistical nor the cosmogonic hypothesis of gap formation can account for these new observations. Scholl²³ has summarized several strong arguments against the collisional hypothesis. We have shown (details will be published elsewhere) that the present distribution of asteroidal semimajor axes can be used to deduce the present semimajor axis of Jupiter to an accuracy of 1 part in 5,000. Thus, there has been very little change in the orbital period of Jupiter since the time of formation of the present gaps. This observation eliminates the otherwise interesting suggestion of Torbett and Smoluchowski²⁴ that the observed gaps were formed by resonance sweeping at the time of the dispersal of the accretion disk. We conclude, therefore, that the gaps have been formed by the gravitational action of Jupiter on individual asteroids and that gap formation has probably continued throughout the lifetime of the Solar System.

The structural differences of the 3:1 and 2:1 gaps suggest that more than one dynamical process may be in operation. In both cases, the libration regions are almost clear of asteroids. However, in the case of the 3:1 resonance, but not that of the 2:1, the gaps in the plots of number density, $\langle e \rangle$ and $\langle \sin \frac{1}{2}I \rangle$ against $\langle a \rangle$ are much wider than the libration widths (details of the 2:1 plots, and those for other resonances, will be published elsewhere). It would appear that some asteroids with circulating resonant arguments have been removed from the vicinity of the 3:1 resonance.

Recent work by Wisdom²⁵ has shown that that region in the asteroid belt where first-order resonances overlap is a region of orbital instability: quasi-periodic orbits cannot exist there. This region lies just outside the orbits of the Hilda group at the 3:2 resonance and is almost devoid of asteroids. However, it is possible that this region was depleted of material by tidal torques exerted by Jupiter in the primitive accretion disk and thus that asteroids failed to form there²⁶. The region of reson-

ance overlap that we discovered lies within the main belt and we have no reason not to believe that that region in $a-e$ space, which corresponds to the region of resonance overlap, and which is now almost devoid of asteroids, was, initially, as well-populated as any other region in the main belt. Thus, the observed depletion and the associated observed trend in the mean eccentricities are strong evidence of post-formative orbital evolution in the asteroid belt.

Goldreich²⁷ has commented that near each resonant ratio of mean motions there are several possible exact resonances (see our Table 1 and our equation (3)). In the asteroid belt, the rates of motion of the pericentres and the nodes $\dot{\omega}$ and $\dot{\Omega}$, are determined by the comparatively weak gravitational attractions of Jupiter and the other planets and are very low ($\sim 2 \times 10^{-4}$ rad yr⁻¹). Hence, in the asteroid belt, these exact resonances overlap and this could lead to chaotic orbital evolution and gap formation. However, for satellites moving about a markedly oblate planet, $\dot{\omega}$ and $\dot{\Omega}$ can be much higher and if the orbital eccentricities and inclinations are low and the satellite-planet mass ratios are small, then the exact resonances may be well separated. We calculate that this separation clearly occurs only in the satellite system of Saturn. It is striking that the saturnian system is virtually saturated with exact first-order resonances. For the uranian system, which is devoid of exact resonances of any kind, we find that the dynamical oblateness J_2 is so low and the satellites are so distant from the planet that, with the observed orbital eccentricities, the exact low-order resonances would overlap. We add that $\dot{\omega}$ and $\dot{\Omega}$ are also very small for planetary orbits and that again the exact low-order resonances would overlap. For this reason, we would not expect any low-order resonances amongst the planets to have long-term stability.

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Effect of wind scouring on climatic records from ice-core oxygen-isotope profiles

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Removal of winter snow by wind explains why the annual mean oxygen-isotope ratio at an ice divide is 2.5% less negative than it is 1.2 km downslope. Cores from two sites on an ice cap, Ellesmere Island, Canada, show past variations in the difference, depending on where the ice originated relative to the scoured zone. Some ice-core climatic records may need correction for scouring.

THE relatively shallow ice caps in the Canadian Arctic islands can provide climatic records comparable in length with those obtained in Greenland and Antarctica, although such records are much less detailed because the oldest ice is confined to the

lowest few metres. However, it is feasible to drill more than one hole in shallow ice caps to check the common assumption that a single core provides a climatic record representative of a wide area. The lowest 5% of the Devon Island cores¹, from

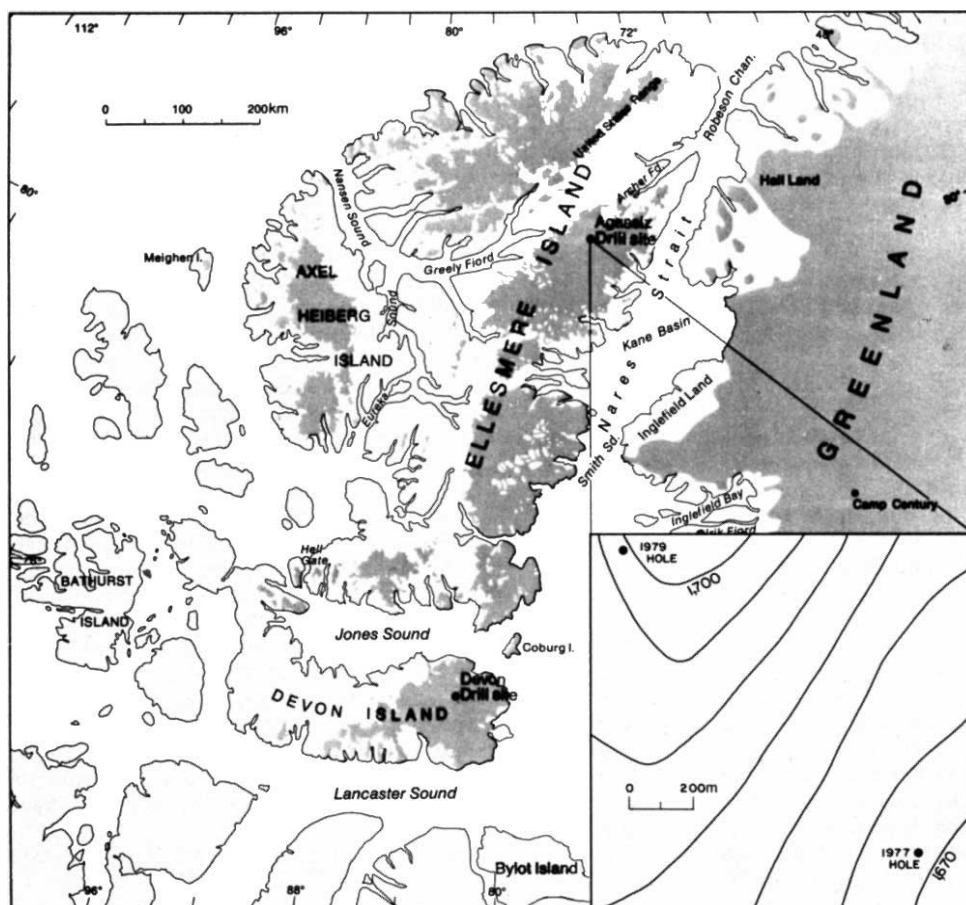


Fig. 1 Location map and map of surface in borehole area showing Holes 77 and 79. Contours are in metres above sea level.

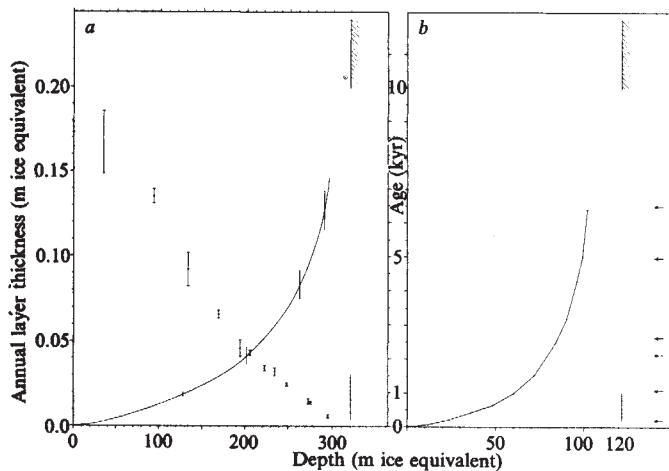


Fig. 2 *a*, Core 77: measured annual layer thicknesses and time scale. Error bars, 2 s.e. in length, were obtained from power-spectral analysis. *b*, Core 79: time scale based on flow model with positions (arrows) of dated volcanic horizons used to check the ages. The circled points denote the position of the sudden δ step at the end of Wisconsin in the two cores.

sites 27 m apart, showed important differences, because the rough bed topography had disturbed the ice flow in the layers above it, producing gaps, and possibly also duplications and inversions, in each record. The present study of two cores 1.3 km apart shows, for the first time, that removal of winter snow by wind can also significantly distort the climatic record.

The ice cap and boreholes

An ice cap, roughly 16,000 km² in area, covers the central part of Ellesmere Island between latitudes 79° 45' and 81° N. Its surface topography is complex and there are nunataks even in the highest parts. The ice cap is drained by glaciers that penetrate the surrounding mountains; the larger ones reach sea

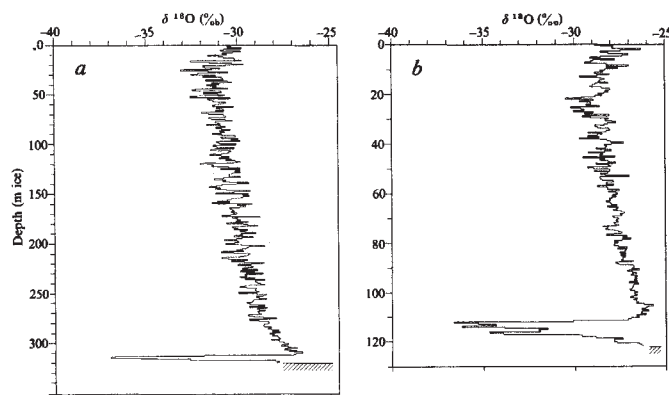


Fig. 3 Oxygen-isotope ratio $\delta^{18}\text{O}$ versus ice-equivalent depth: *a*, Hole 77. *b*, Hole 79. The A77 δ s are 1 m averages and the A79 δ s are 50 cm averages.

level. The northern part—the Agassiz ice cap—is where two cores were recovered in 1977 and 1979 (A77 and A79, see Fig. 1). Hole 77 (338 m deep) is ~1 km downslope from a local ice divide at an elevation of ~1,700 m. Hole 79 (139 m deep) was supposed to be at the top of the flow-line, but subsequent measurements have shown that it is ~200 m down the opposite slope. Down-borehole photography and television confirmed that both holes reached the base of the ice². Surface and basal temperatures are -24.2 and -16.7 °C respectively at Hole 77 and -22.4 and -19.0 °C at Hole 79. Annual precipitation is 0.175 m yr⁻¹, ice equivalent. Summer melting, as shown by ice layers in the firn, is slight, ~3% of the annual precipitation on average.

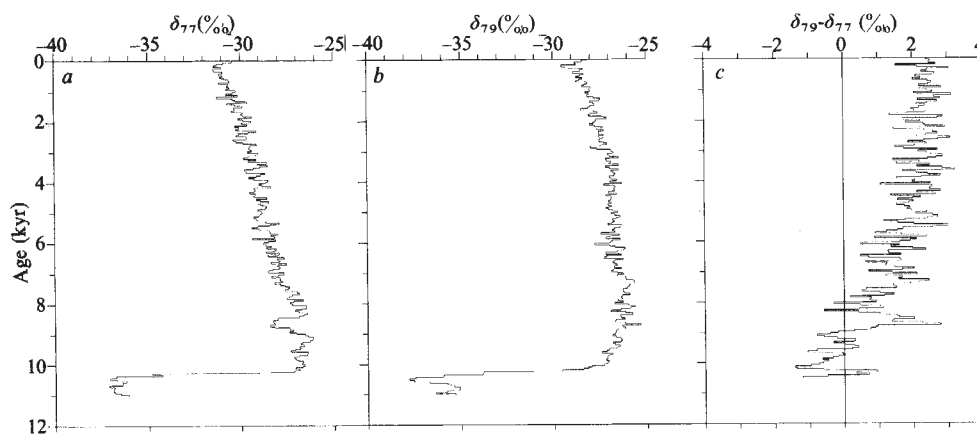


Fig. 4 *a*, Oxygen-isotope ratio $\delta^{18}\text{O}$ versus time back to 12,000 yr BP, Hole 77. *b*, δ Hole 79. *c*, Difference, $\delta_{79} - \delta_{77}$. Data are presented in 50-yr averages.

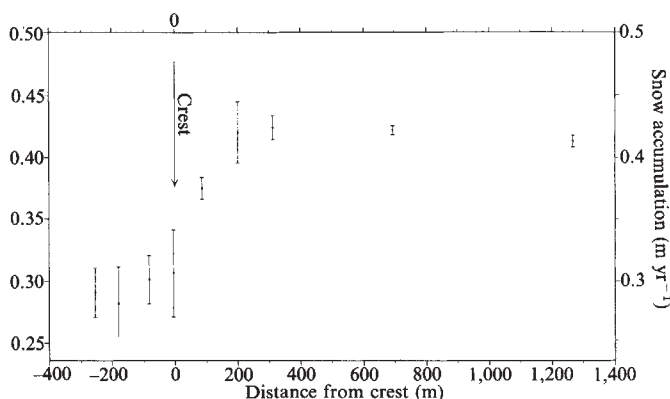


Fig. 5 Snow accumulation rate (4-yr average) versus distance from ridge. The 4-yr averages are based on measurements made on 45 stakes comprising the strain and accumulation net. The network enclosed most of both flow lines. Estimated errors are indicated by bars and include areal and temporal variance.

Time scale

The Hole 77 core was dated by measuring annual layer thicknesses at various depths. Because the precipitation rate is low, seasonal variations in oxygen-isotope ratio are smoothed out by diffusion after ~ 25 yr. However, as on Devon Island, the spring peak in concentrations of soluble and insoluble impurities can be distinguished until the layers become too thin to sample³. At each depth, samples were divided into about 10 horizontal sections per estimated year thickness. About 15 consecutive years were sampled at 12 depths spanning 0–7,000 yr BP. Concentrations of insoluble particles (diameter $1 > \mu\text{m}$), Ca, K and Na, electrolytic conductivity and, in some cases, acidity were measured. Two least-squares determined straight lines were fitted to the graph of annual layer thickness versus depth; the dividing depth was 170 m. The age of ice at each depth was calculated by integrating, from the surface to that depth, the reciprocal of the annual layer thickness. Figure 2*a* shows layer thicknesses and the time scale. Scatter about the regression lines and inaccuracies in measuring layer thicknesses are the only sources of error in this time scale; thus we estimate that it is accurate to $\pm 10\%$ back to 7,000 yr BP.

Because Hole 79 is only 139 m deep, the depth where layers become too thin to sample corresponds to much younger ice than in Hole 77. We therefore estimated ages from a flow model. Raymond⁴ has used finite-element modelling to compute the variation with depth of the horizontal and vertical

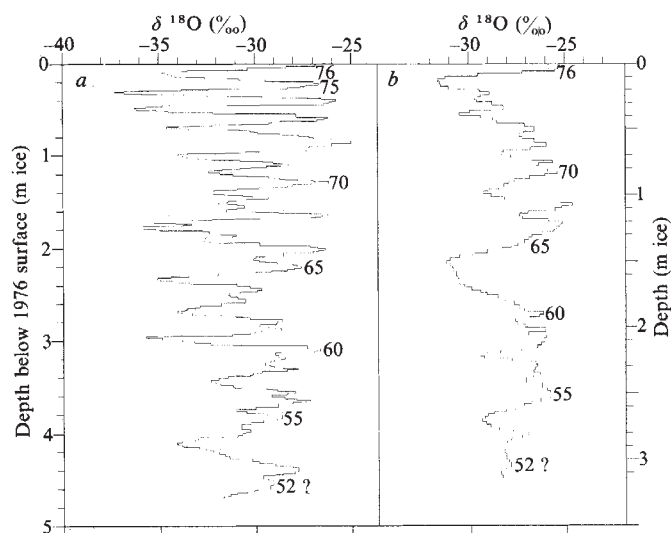


Fig. 6 Seasonal variations in oxygen-isotope rate over the period 1952–76 at: *a*, Hole 77; *b*, Hole 79.

components of velocity near an ice divide, a problem that has not been solved analytically. If the ice cap is in a steady state, the vertical velocity component, expressed as the distance an ice particle moves downward in 1 yr, is equal to the annual layer thickness at each depth. Integration of the reciprocal of the layer thickness then gives the time scale as before. The vertical velocity component at the surface, which has to be known, was obtained by laser measurements of the shortening of the borehole in 2 yr. The value, reduced to ice equivalent by using the measured density–depth relation, is equal to the mean annual accumulation rate measured over the past 4 yr; this suggests that the ice cap is close to a steady state at present. The time scale (Fig. 2*b*) was checked by comparison with highly acid volcanic horizons in the core, detected by the scratch conductivity technique⁵. Each horizon was identified by comparison with the A77 core. The six largest peaks in each were synchronous within the errors of the A77 time scale. Furthermore, five of the six were synchronous with the five largest acid peaks in the Camp Century ice core⁶ back to 6,500 yr BP. We estimate that the A79 time scale is accurate to $\pm 10\%$ back to 6,500 yr BP.

The large abrupt change in oxygen-isotope ratio near the bottom of each core provides another reference horizon. This 'step', which coincides with a sudden change in the concentration of microparticles (many more particles in the older ice) has also been found in the Devon Island^{1,3} and Camp Century⁷ cores and at Byrd Station^{7–9}, Dome C^{10,11} and Vostok¹² in

Antarctica. This horizon represents the end of the last glaciation; we choose the date given by the Camp Century time scale, 10,500 yr BP, beyond which our cores are not dated.

Oxygen-isotope profiles

The usual correlation between $^{18}\text{O}/^{16}\text{O}$ ratios relative to SMOW, (δ) and temperature must be made with caution because δ will also change if the source area of precipitation changes, if the ice thickness changes with time, or if the ice at depth originates upslope from the drill site¹³. Drilling near an ice divide, as in the present study, eliminates the last problem provided that the position of the divide has not changed. However, the A77 and A79 δ profiles (Fig. 3 against depth and Fig. 4 against time) show striking differences. The difference shows no significant trend beyond 8,000 yr BP, and these fluctuations may result largely from inaccuracies in the ages. An exception is the step change in the Hole 77 value from about 8,800 to 8,400 yr BP for which we have no explanation. The values start to diverge at ~8,000 yr BP. If short-period fluctuations are disregarded, the difference increases steadily to 2.5% at about 3,000 yr BP and then stays constant. Hole 79, where the surface is ~25 m higher than at Hole 77, is the isotopically 'warmer' site. We believe that the removal of winter snow by wind can explain these observations, as we think the initial undisturbed snowfall has the same δ value at both sites.

Evidence for wind scouring

Figures 5 and 6 present the evidence for scouring. Accumulation on the A79 side of the crest is only ~65% of that at A77, and remains constant for ~10 km south of A77. The near-surface values of δ at A77 show the normal pattern of seasonal variations, whereas at A79 the seasonal cycles are poorly defined and, in particular, the 'cold' peaks (most negative δ) are truncated. If the 'coldest' 35% of the Hole 77 record is numerically removed, its mean δ is changed from -30.3‰ to -27.7‰ which is equal to the A79 value. Some of the 'warm' peaks in the A79 record are also reduced in size. This shows that while scouring occurs mainly in the winter, it is not confined to that season. On the other hand, more rapid diffusion at A79 as a result of the lower accumulation rate may also help to reduce the summer peaks.

We believe that katabatic winds in combination with general circulation cause the scouring. Holmgren¹⁴ found that these winds develop on Devon Island ice cap when there is a strong temperature inversion immediately above the surface. This occurs when the sky is clear, particularly at night. On relatively steep slopes, the wind velocity increases and exceeds the critical value at which it can remove surface snow. Scouring also occurs at well defined ridge crests because they cause turbulence in the general circulation winds. Conditions for scour prevail near the ridge crest of the Agassiz ice cap and down the steep slope north-west of A79. We expect that katabatic winds occur more often in winter, when snow grains are smaller than in summer and take much longer to compact; they are, therefore, more easily blown away. Thus most of the scouring occurs in winter.

Accumulation measurements show that the borehole area (Fig. 1) can be divided into three zones: (1) a scoured zone extending 5 km north-west from the crest where mean accumulation is ~0.115 m ice yr⁻¹; (2) a non-scoured zone extending south-east for several kilometres, starting ~250 m east of the crest where accumulation is about 0.175 m ice yr⁻¹; (3) a transition zone between the two in which accumulation increases roughly linearly with distance from the crest. A77 lies in zone (2) and A79 in zone (1).

The difference between the oxygen-isotope profiles (Fig. 4) can be explained in terms of where the ice in each core originated. We confine discussion to the period since 10,000 yr BP, and assume that the present pattern of scouring has persisted since that time and that the surface crest is the flow divide. Because the scoured zone extends to the crest on the A79 side, all the ice (younger than 10⁴ yr) in that core originated in the

scoured zone. We postulate that in A77, ice older than 8,000 yr originated on the crest, ice between 8,000 and 3,000 yr old originated in the transition zone, and ice younger than 3,000 yr originated in the non-scoured zone. This would explain the observations: values of δ in the two profiles start to diverge at 8,000 yr BP, the difference increases steadily from 8,000 to 3,000 yr BP and remains constant thereafter.

Although we believe that this explanation is correct, there is a difficulty. We assumed that the flow-lines are perpendicular to the surface contours, as measurements at A77 confirm, and calculated the travel times of the ice using present ice thicknesses and balance velocities. (The balance velocity is that which, with present ice thickness, will just carry off the present accumulation and so maintain the ice cap in a steady state.) Calculated times to the present position of A77 were 1,500 yr from the edge of the transition zone and 4,200 yr from the crest. Moreover, the slope of the graph of annual layer thickness (Fig. 2a) changes at a depth of ~170 m which is equivalent to an age of 1,700 yr, which implies that only the ice younger than this age originated in the scour free zone. The conflict between these dates of ~1,500 and 3,000 yr BP needed to explain the isotope difference suggests that accumulation rates, ice thicknesses and the position of the scouring zone may have varied in the past. Numerical modelling of ice flow in the area should clarify this.

The Holocene climatic record

Because of scouring, these profiles are of limited value as climatic records. However, the A77 record should represent true climatic changes for at least the part 1,500 yr and possibly the past 3,000 yr. The trend in δ from 3,000 yr BP to the present, as far as it can be represented by a straight line, is a decrease (cooling) of 0.058‰ per 100 yr, which is close to the value of 0.051‰ for Devon Island and A79. This represents a cooling of 3 K in 3,000 yr with the normally-used relation between δ and temperature¹³. Because scouring biases the A79 δ record towards summer temperatures, the agreement with the A77 record suggests that trends in summer temperatures and mean annual temperatures over this period have been similar. The positions and amplitudes of warm and cold peaks in the Devon Island and A77 records for this period are also in fair agreement. For example, all three records show the Little Ice Age, starting around AD 1650 and a pronounced warming starting shortly after 1900. During the Little Ice Age, the difference between the δ values in Holes 77 and 79 is reduced which might indicate a reduction in scouring. However, we suggest that the cooling in summer was greater than the decrease in mean annual temperature. A complete numerical modelling of flow should produce a true climatic record by correcting for the effect of scouring. The pre-Holocene part of the cores is discussed elsewhere¹⁵.

Scouring on other ice caps

As already mentioned, scouring occurs on parts of the Devon Island ice cap. Koerner and Russell¹⁶ concluded that, on the north-west side, winter snow is scoured from some areas and deposited in others; thus explaining the unexpectedly high variation in δ of surface samples. They also predicted that "if a core-site has moved in the past relative to the crest of an ice cap, and is close to it, any trend in δ with time may be attributable to the wind-scour effect rather than climatic change". However, the Devon Island cores should not be affected by scouring. Measurements of accumulation rate and seasonal variations of δ in near-surface samples show that at present there is no scouring or deposition in the borehole area. Moreover, because the surface crest coincides with a prominent bedrock ridge, we think that migration of the crest is unlikely.

In 1981, a 2,037-m borehole was drilled through the Greenland Ice Sheet at Dye 3 (ref. 17). This station is ~35 km east of the crest. Reeh and others measured accumulation rate and δ of surface samples on a 60 km traverse. Accumulation

increased from 0.31 m yr^{-1} near the crest to about 0.43 m yr^{-1} at the station. The value of δ had a minimum about 8 km upslope from the station and the rate of decrease in δ with increase in elevation in the vicinity of the station was five times the normal value¹⁸. These observations suggest that winter snow is scoured from near the crest and deposited a few kilometres upslope from the station. The high variance of accumulation rate around the station suggests deposition or scouring there. Moreover there are large surface undulations and therefore,

changes in slope in the vicinity¹⁸. We believe that the Dye 3 oxygen-isotope record will have to be corrected for scouring effects and this may not be simple.

At Byrd Station, Antarctica, the surface has undulations with wavelengths of a few kilometres; less snow accumulates on the crests than in the troughs¹⁹. It has been realized that these variations in accumulation may affect the calculated time scale for the core. We suggest that the climatic record may also need correction for scouring.

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Role of fimbrin and villin in determining the interfilament distances of actin bundles

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From X-ray diffraction patterns the interfilament distances of native and reconstituted actin bundles, and of purified actin gels subjected to a range of g forces by centrifugation, have been obtained and compared. The results suggest that bundling proteins, such as villin or fimbrin, cross-link actin filaments around a minimum distance probably set by electrostatic repulsion.

THE apical end of intestinal epithelial cells is lined by a brush border made up of densely packed microvilli with an underlying meshwork of filaments called the terminal web. Microvilli are typically 1–2 μm long and 100 nm in diameter, and contain a core bundle of filaments that are attached to the overlying plasma membrane by helically arranged¹, periodically spaced membrane linkages^{2,3}. Triton-demembrated microvilli contain proteins of molecular weights (MWs) 110,000, 95,000 (villin), 68,000 (fimbrin), 42,000 (actin) and 16,500 (calmodulin)^{4–6}. Biochemical and electron microscopic studies have suggested that both villin and fimbrin are in the core bundle⁴, while calmodulin and the 110,000-MW protein may comprise the membrane linkages^{4,7}. Although actin bundles have been constructed *in vitro* with either purified villin or fimbrin^{5,6,8–11}, it is not known whether both proteins have the same cross-linking function in microvilli as they differ in molecular weight and some of their actin binding properties.

Our understanding of actin filament structure has come mainly from the analysis by optical diffraction and image reconstruction methods of electron micrographs of negatively stained specimens^{12–16}. More recent studies have focused on the organization of actin bundles in non-muscle cells^{17–20}. However, as has been shown by comparative X-ray and electron microscopic studies of muscle^{21,22}, specimens prepared for electron microscopy experience changes in their structure as a result of fixation, dehydration and embedding. These processes cause shrinkage in the structure and induce uncertainty in the quantitative information obtained from electron microscopy.

This problem is avoided in the X-ray diffraction analysis of hydrated specimens. Therefore we chose this method to study

the brush border cytoskeleton and to establish a quantitative basis for future structural investigations. We obtained X-ray patterns from isolated brush borders and microvilli, from which we measured the interfilament separation (centre-to-centre distance) in their actin core bundles. As these were the first X-ray patterns from an actin-based non-muscle cytoskeleton, the reflections were compared with those from actin bundles reconstituted *in vitro*, and also with F-actin gels.

Thin sections of microvilli and reconstituted actin bundles

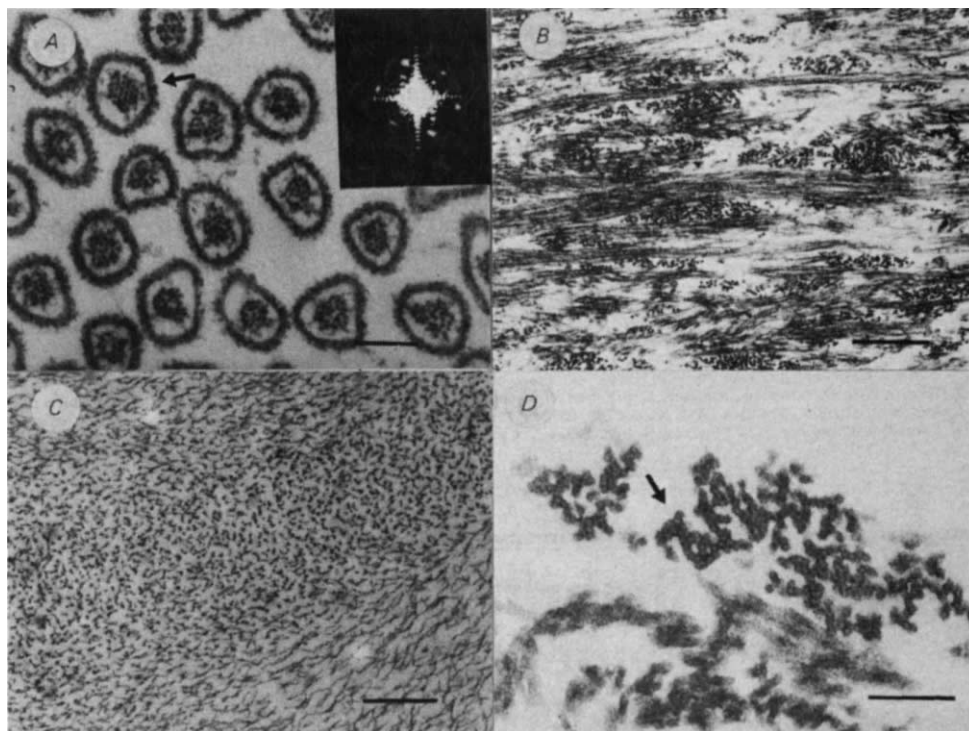
Cross-sections through brush border microvilli (Fig. 1A) show a central core of bundled microfilaments, which are attached to the surrounding membrane linkages. In intact brush borders the microvilli are packed in a fairly regular hexagonal lattice, and the microfilament bundles are restricted in their approach by their surrounding membranes, which can be selectively removed by treatment with detergent. Isolated microvilli display in pellets quasi-hexagonal packing in restricted domains, but many randomly orientated microvilli are also present. Filaments within a bundle (arrow) can also be found hexagonally arranged, as shown by optical diffraction (inset).

F-actin bundled by fimbrin and/or villin exhibits quasi-crystalline domains comprising several dozen filaments which are nearly parallel within a given domain, although the domains themselves are randomly orientated (Fig. 1B). Because bundles do not pack very evenly, the separation between filaments is more uniform within a bundle than within the pellet as a whole.

By contrast, pellets of F-actin contain large areas of widely dispersed filaments with no obvious order visible in thin sections (Fig. 1C). On the other hand, 50 mM Mg^{2+} induces the formation of paracrystals^{23,24} which differ greatly in their organization

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Fig. 1 Thin sections through pellets of brush border microvilli and actin preparations. **A**, cross sections through brush border microvilli isolated from chicken intestine epithelial cells according to previously described methods^{4,5}. **B**, section through a pellet of F-actin cross-linked with fimbrin. **C**, section through a pellet of F-actin polymerized without bundling proteins. **D**, cross-section through Mg^{2+} induced paracrystals. **A**, brush borders are composed of densely packed microvilli, each consisting of an outer membrane and an inner core. The inner core contains the proteins actin, fimbrin and villin, which are organized into a bundle of filaments. The linkages between the membrane and the core bundle are faintly seen in projection. As measured from thin sections, the diameter of microvilli varies between 100 and 150 nm, and the space between adjacent microvilli is about 10 nm. Each bundle contains from 10 to 40 filaments, which are seen as dots in cross sections. An optical diffraction pattern (inset) from one of the core bundles (arrow) shows



that the filament packing is roughly hexagonal with an interfilament separation between 10 and 12 nm. Scale bar, 100 nm. **B**, the pellet contains paracrystalline domains of bundles whose long axis are parallel. The layered appearance of the pellet is a result of the random orientation between neighbouring domains. This pattern is also observed in pellets of F-actin cross-linked with villin, a mixture of villin and fimbrin, or with Mg^{2+} induced paracrystals. Scale bar, 250 nm. **C**, actin filaments are dispersed into large areas of filaments with similar orientation. Unlike actin bundles, boundaries between domains of different orientation are not abrupt but very gradual. Scale bar, 250 nm. **D**, these specimens differ from both the F-actin pellet and the bundles formed with fimbrin and/or villin in several respects. Adjacent filaments are very closely spaced and seem to be touching one another. Thus their interfilament separation is much smaller than in the other preparations. In addition, they form linear and branched ribbons (arrow) which cannot be described by quasi-crystalline order (compare **D** with **A** and **B**). Scale bar, 100 nm.

Methods: Actin (for **B**) was isolated from chicken breast muscle⁴⁰, filtered through a Millipore filter (0.45 μm pore size), and used fresh or stored at $-70^{\circ}C$. Villin and fimbrin were purified using a modified procedure of previously published methods^{5,9} (L.K.H. and K.W., in preparation) and stored in 50% glycerol at $-20^{\circ}C$. Actin was polymerized in bundling buffer (75 mM NaCl, 1 mM $MgCl_2$, 1 mM EGTA, 3 mM NaN_3 , 10 mM PIPES pH 7.0) at a final concentration of 20 μM . Villin or fimbrin were dialysed into bundling buffer and mixed with F-actin at molar ratios of cross-linker to actin of between 0.4:1 and 1:1. The fimbrin bundle shown in **B** was assembled at a 1:1 ratio with actin, which gives a 1:10 molar ratio in the pellet. This ratio is close to that measured in the microvilli. Proteolysis of fimbrin was detected in some fimbrin-actin mixtures, which during the experiments had no detectable effects on bundle formation. After 8 h the bundles were negatively stained with 1% uranyl acetate, and examined in the electron microscope, before centrifugation in an Airfuge (Beckman) at 160,000g for 30 min. The bottom of the tube containing the pellet was cut out, fixed in tannic acid and glutaraldehyde, and processed for thin sectioning as described before¹. Paracrystals (for **D**) were formed from a 20 μM solution of F-actin by the addition of $MgCl_2$ to 50 mM. The solution immediately became turbid. After 1 h at room temperature, the solution was centrifuged for 30 min in the airfuge as described above. To ensure adequate preservation of the spatial arrangement of actin filaments in the paracrystal, $MgCl_2$ was added to 50 mM to the fixative.

from the other actin preparations. Here actin filaments are tightly packed into linear and branched aggregates which are not organized along any regular lattice (Fig. 1D).

X-ray scattering results

Isolated brush borders and microvilli: X-ray patterns from isolated brush borders or microvilli (Fig. 2A,B) display two distinct sets of reflections. There is a pair of arced reflections at Bragg spacings of approximately 36 and 54 nm, superimposed on an intense background (see inset). These arise from a regularly packed array of microvilli whose centre-to-centre distance is ~ 110 nm. The reflections are no longer observed in either demembranated microvilli or any of the reconstituted bundles (Fig. 5). This indicates a loss of regularity in the spacing between the bundles which is clearly seen in thin sections (Fig. 1B). As the separation of microvilli must exceed their outer diameters, which are at least 100 nm, the observed reflections probably represent the second and third orders of the basic repeat. We cannot deduce the separation nor the lattice of the microvillar paracrystals accurately from the present data but it is remarkable that such sharp reflections can be observed with isolated cell organelles. Although the axial repeats for both the

membrane linkages and the actin filaments are on the order of 36 nm, these structures cannot be responsible for the small angle reflections as their contrast would be too low to be detected.

The second set of reflections is at Bragg spacings of 12 and 6.7 nm. As shown below, they are characteristic of an hexagonal array of actin filaments separated by 13.8 nm. This is the first direct determination of the actin interfilament separation in hydrated non-muscle cytoskeletons. The regularity of the bundle is revealed by the weak but visible second order reflection at 6.7 nm. The ratio of positions is 1.78 which is close to the expected value (1.73) for hexagonal packing (orders (1,0) and (1,1)).

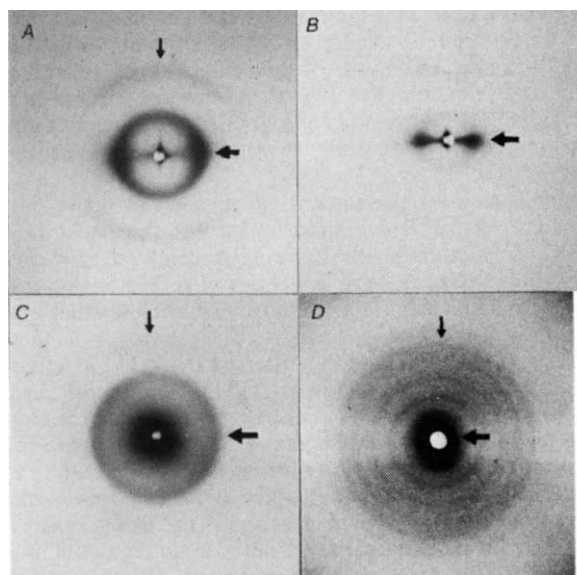
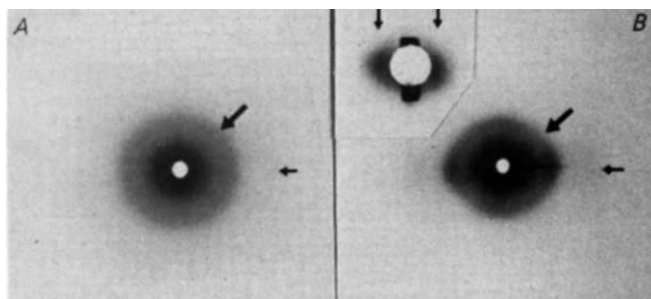
Gels of F-actin polymerized *in vitro*: The scattering patterns of solutions of F-actin were studied to confirm whether the main equatorial reflections observed in isolated microvilli were contributions from the actin packing. We examined whether experimental parameters such as centrifugal force or ionic strength could change the spacings of the reflections. When skeletal muscle actin (20 μM) was polymerized in 75 mM NaCl or 2 mM $MgCl_2$, there was no noticeable increase in light scatter from the actin solutions, an indication that the filaments were

Fig. 2 X-ray patterns from isolated brush borders and microvilli. *A*, brush border preparation (film 46). *B*, microvilli preparation (film 40). *A*, outside the beam stop (centre) one observes a disk of central scatter, which extends to 35 nm and on which is superimposed a faint circular reflection at about 36 nm. Because of disorientation it can only be seen by eye, but the same reflection is seen in patterns from microvilli. The dominant reflection is a ring at 12 nm (arrow) followed by a very weak ring at 6.7 nm (small arrow). The ratio of positions is 1.78, indicating that they can arise from filaments with a nearly hexagonal packing. The separation of the filaments is $2/\sqrt{3} \times 12 = 13.8$ nm. *B*, this film shows the same basic features as the previous one, except that the peaks are more clearly visible and there is some orientation. Superimposed on the background at very small angles are two reflections at approximately 36 and 54 nm, centred on the equator (error in measurement ~20%). This is seen more clearly in a less exposed film (inset) which shows the position of the 54-nm reflection (arrow; the 36-nm reflection could not be adequately reproduced in the photographs). The 54- and 36-nm reflections index on a repeat of ~110 nm, which is about the diameter of a microvillus. As with isolated brush borders, there is a dominant reflection at 12 nm (arrow) and a weaker one at 6.7 nm (small arrow), which both arise from the core bundle. Although these reflections are centred on the equator, they show more angular disorientation than those at the lower angles. This can be explained by assuming that the very low angle reflections are mainly from the interference between microvilli packed in domains roughly parallel to the capillary axis, while the 12 and 6.7 nm reflections come from the interference between actin filaments within core bundles of all microvilli, including those dispersed in the pellet in random orientations. Model calculations show that the molecular scattering has little influence on the lattice scattering of actin filaments, which are approximated by solid cylinders, but it influences the peak positions of the microvillar lattice since microvilli are hollow cylinders with a central core. This probably explains why the microvillar reflections are not exactly on the positions expected for an hexagonal lattice.

Methods: The patterns were obtained using a rotating anode X-ray generator (Elliott GX 13, copper K α line, wavelength 0.154 nm) equipped with a double mirror camera (mirror length 20 cm) built by Dr A. Harmsen⁴³. The specimen-to-film distance was 56 cm, and the useful range of spacings was between 60 and 4 nm (spacings are quoted in terms of $1/S$ where $S = (2 \sin \theta)/\lambda$). Higher angle patterns were obtained on a GX6 generator (Elliott) equipped with a double mirror camera and a specimen-to-film distance of 9 cm (spacings range 8 to 0.5 nm). The specimens were pushed into a thin-walled quartz capillary tube (1 mm diameter). For soft pellets of polymerized F-actin some orientation could be achieved by pushing the specimen to and fro⁴⁴, but specimens containing paracrystalline bundles could not be oriented this way. The degree of orientation was assessed under the polarizing microscope before taking an X-ray photograph. Exposure times ranged from a few hours to 2 days, using Agfa Osray T4 or Ceaverken Reflex 25 X-ray films. The photographs were scanned on an Optronics P-1000 densitometer linked to a Norsk Data Nord-100 computer. Optical densities were integrated within arcs whose angular range was matched to the disorientation of the specimen. The integrated scattering intensities were displayed on an x-y plotter, and peak intensities and positions determined from the traces.

Fig. 3 X-ray patterns from F-actin preparations. *A*, F-actin centrifuged at 220,000g (film 20). *B*, F-actin centrifuged at 110,000g (film 21). *C*, Mg²⁺-induced F-actin paracrystals (film 11).

Methods: F-actin, or Mg²⁺ induced actin paracrystals, were prepared as described in Fig. 1 legend. The actin solutions were centrifuged at forces varying between 110,000g and 315,000g for 1 h in a type 65 rotor (Beckman). *A*, this specimen formed a hard pellet. The pattern shows a strong first order reflection at 10.8 nm centred on the equator and weak near-meridional reflections at 5.1 and 5.5 nm. The background scatter is very low compared with the previous figure, indicating the homogeneity of the specimen. Unlike with the microvillar core bundle we do not observe higher orders of the interparticle interference suggesting that the order of the packing is lower than in the microvillar core bundles. It is thus not possible to deduce the type of packing with certainty, but assuming hexagonal order between nearest neighbours, the separation between filaments is different in the two cases. The near meridional reflections arise from the actin filament substructure, mainly from the spacing of subunits along each of the two actin strands (5.5 nm). *B*, this specimen was a soft, almost liquid pellet and could be partially oriented in the capillary. Only one major reflection at 16 nm could be observed centred on the equator. The equatorial streak at smaller angles probably arises from a heterogeneous population of filaments with larger separations. Other reflections are too weak to be observed. *C*, the main difference with all other patterns shown so far is that the first reflection is at a spacing of 6.9 nm (arrow) and the second at 4.4 nm (small arrow). The interfilament separation is estimated to be near 7.0 nm. The diameter of the actin helix is around 8 nm or more, depending on how the actin molecule is oriented relative to the helix axis (see ref. 45). Thus adjacent filaments in a paracrystal must touch or even slightly interlock with each other. The heterogeneity of the specimen shows up in the strong central scatter (compare with *A* and *B*).



published, with the differences explained by the partial orientation of our samples. In contrast, when centrifuged at 110,000g the actin pellet is considerably softer than those centrifuged at higher g forces. Its X-ray pattern (Fig. 3B) shows an arc centred on the equator at a Bragg spacing of 16 nm. The equatorial intensity extends towards the beam stop which is an indication that the distances between actin filaments are often larger than 16 nm.

The reflections at 5.5 nm arising from actin filament substructure were not affected by centrifugation indicating that the

largely dispersed. By contrast, when F-actin was incubated in 50 mM MgCl₂ there was an immediate increase in light scattered.

A typical X-ray pattern (Fig. 3A) of F-actin polymerized in 75 mM NaCl and centrifuged at 220,000g shows a circular reflection at a Bragg spacing of 10.8 nm, centred on the equator and near meridional reflections between 5.1 and 5.5 nm. The meridional reflections are characteristic of the internal structure of actin filaments²⁵⁻²⁸ and serve as calibration standards for the small angle reflections. This pattern is similar to those previously

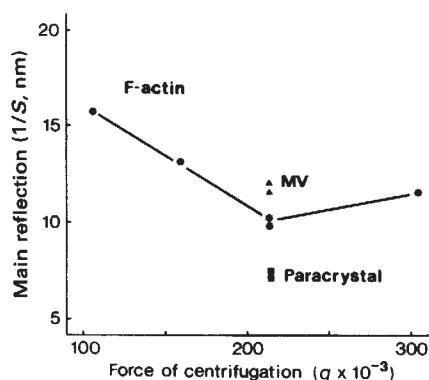


Fig. 4 Dependence of the spacing of the interparticle interference peak on centrifugal force. For comparison the spacings obtained from microvilli (MV) and Mg^{2+} paracrystals centrifuged at 220,000g are also shown. The dependence of the Bragg spacing on the experimental manipulation of the interfilament separation, that is, by addition of Mg^{2+} or centrifugation, proves that the low angle peaks observed in the actin-containing preparations indeed arise from the packing of filaments rather than their substructure. **Methods:** Actin filaments were prepared as described in Fig. 1 and centrifuged for 1 h in a type 65 rotor (Beckman) at 110,000g, 155,000g, 220,000g and 315,000g (4 °C). Bragg spacings vary from 16 to 11 nm. This minimum value cannot be decreased by either harder centrifugation or high ionic strength (up to 0.8 M NaCl).

filaments themselves did not undergo major changes in different conditions. In one specimen we observed a weak equatorial reflection at 23 nm. A similar reflection was reported by Vazina and her colleagues²⁹, who interpreted it in terms of the actin filament substructure. This interpretation is probably not correct, as pointed out earlier³⁰. In control experiments with acid-denatured actin (pH 4.5), we observed a disk of reflection extending to 23 nm, but not 12- or 5.5-nm reflections. It is therefore possible that the other specimen also contained some denatured material. We do not have a structural interpretation for this reflection.

The actual value for the separation between filaments can be deduced from the Bragg spacings only if the packing is known. We can determine the packing in oriented filaments from an X-ray pattern if there is a series of reflections on the equator. For example, solutions of rod-like particles, which are nearly hexagonally packed, give rise to a characteristic sequence of reflections corresponding to the orders (1,0), (1,1), (2,0), etc. Examples are tobacco mosaic virus (TMV)³¹, muscle³² and bacteriophage³³. In contrast to X-ray patterns of isolated microvilli the actin gels did not show higher orders of the equatorial reflections that would allow us to distinguish between different types of packing. For hexagonally packed actin gels, the particle separation would be $1.154 \times 1/S$, where S is the Bragg spacing in nm^{-1} , while for more randomly ordered actin filaments, the separation would be closer to $1/S$ (refs 30, 34). Because of the absence of higher order reflections in our patterns from actin gels, the uncertainty of our determination of the interfilament separation is approximately 15%.

As shown by Spencer³⁰, the actin concentration can be calculated from the spacing of the equatorial reflection. Although we have not formally determined the actin concentration in the pellets, we have varied it by centrifuging the gels at different g forces. In Fig. 4 it can be seen that a minimum spacing of 11 nm is reached at 220,000g, and cannot be decreased by increasing the g force. Assuming 28 actin monomers (MW 42,000) per 77-nm repeat, we calculate an actin concentration (c) of $2.2 \times 10^4 S^2$ (where c is in $mg\ ml^{-1}$ and S in nm^{-1}). Thus, at $S = 1/16\ nm$ the concentration of F-actin in the pellets would be $86\ mg\ ml^{-1}$ (2 mM), and at $S = 1/12\ nm$ it would be $153\ mg\ ml^{-1}$ (3.6 mM). The latter value represents the concentration of F-actin in the microvillar core bundle.

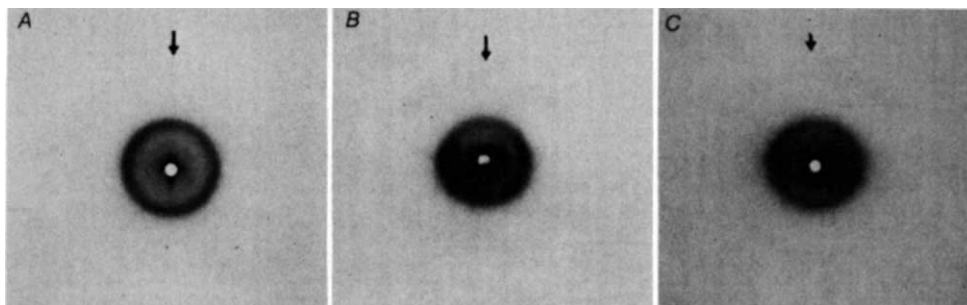
It is likely that the minimum separation is determined by the electrostatic repulsion of actin filaments acting against the compression by centrifugation. In the usual buffer conditions (see Fig. 1) the Debye screening parameter is $\sim 1\ nm$. It can be decreased to 0.4 nm by raising the salt concentration to 0.8 M NaCl. However, even then the minimum separation was around 11 nm, in striking contrast to the effects of Mg^{2+} (see below). This is consistent with the very steep rise of the repulsive force, within our range of ionic strengths, when the separation of filaments is reduced below 20% of their diameter³⁵. A quantitative comparison between experiments at different ionic strengths is difficult, however, because high salt influences the sedimentation properties at a given g force, and the electron density contrast between protein and solvent is reduced so as to make the reflections faint even after long exposures.

Mg^{2+} -induced actin paracrystals: Mg^{2+} at 50 mM induces the formation of paracrystalline arrays of F-actin^{23,24}, which results in a >10-fold increase in light scattered between 18° and 160° (P.M., unpublished data). In the X-ray patterns, two prominent peaks at 6.9 and 4.4 nm were observed. There was no preferred orientation in these specimens so that all reflections were uniformly distributed over circles. Interpretation of this pattern is complicated by the possible contribution of non-equatorial reflections, which are enhanced as a result of the interlocking and packing of the filaments. Based on comparisons with published X-ray patterns from oriented actin gels^{23,28} and optical diffraction patterns of negatively stained Mg^{2+} paracrystals^{15,16}, the 6.9 nm peak is probably a combination of the equatorial and first layer lines. A second reflection at 4.4 nm can be assigned to the 5.9 nm pitch of the actin helix, which was seen as a near-meridional reflection in the other actin patterns (see Figs 3A, 5) but in this sample is probably sampled at the first row line positioned at 7 nm. As demonstrated previously by both X-ray and optical diffraction, the 5.9-nm reflection would strongly dominate this region. As there is uncertainty about the packing due to the absence of a second equatorial reflection, the interfilament spacing is between 7 and 8.1 nm. Because cross-sections through paracrystals (Fig. 1D) show a lack of crystalline order, the interfilament spacing in the hydrated specimens is likely to be closer to 7 nm. Error introduced in the measurement of the main reflection due to the presence of a 1st layer line would decrease the value for the interfilament spacing by 1.7%, and can therefore be disregarded. Thus, our measurement for the interfilament spacing is about 20–25% larger than the value (6.0 nm) measured from negatively stained actin paracrystals¹⁴. One possible explanation for this difference is shrinkage during electron microscopic preparation. The calculated local protein concentration in a Mg^{2+} paracrystal is $450\ mg\ ml^{-1}$ (9.5 mM), about 2.6 times higher than found in packed actin gels or actin bundles.

Because there are probably specific interactions between adjacent filaments in a Mg^{2+} paracrystal, leading possibly to a small amount of interlocking as found for TMV³⁶, the interfilament separation cannot be directly used to measure the outside diameter of the actin filament. By modelling it from spheres of diameter 4.6 nm, a value calculated from a MW of 42,000 and partial specific volume of $0.73\ ml\ g^{-1}$ for actin, the outside diameter is estimated to be 8 nm. This is in good agreement with values derived from muscle patterns³⁷. Thus the actin filaments in a Mg^{2+} paracrystal could interlock by about 1.0 nm which is somewhat less than the value deduced from optical diffraction patterns¹³. Comparing the apparent diameters reported for actin in the literature we find 5.5 nm for dried preparations which are presumably strongly interlocking²⁶, 6.0 nm derived from negatively stained images of Mg^{2+} paracrystals¹³, approximately 7.0 nm from hydrated Mg^{2+} paracrystals in which the filaments are probably slightly interlocking (this study), between 8 and 9 nm from X-ray patterns of muscle thin filaments^{37–40}, and $\sim 12\ nm$ for the effective diameter including electrostatic repulsion in solution (ref. 30 and this study).

Due to the higher concentration, the intensity of the reflections arising from actin filament substructures is more

Fig. 5 X-ray patterns from reconstituted actin bundles. **A**, actin mixed with fimbrin at an equimolar ratio (film 43). **B**, actin mixed with villin at a 8:20 molar ratio (film 26). **C**, actin mixed with villin and fimbrin at a 8:20:20 molar ratio (film 42). **A**, this specimen shows a prominent equatorial interparticle interference peak at 11.1 nm which is distributed around a circle because of disorientation. There is a weak ring at 5.5 nm (small arrow) from the actin substructure. We also observe a weak ring at 23 nm (see text). **B**, this pattern shows the main interference peak at 12 nm and a weak reflection at 5.5 nm (small arrow). As in the previous sample there is little orientation. **C**, the pattern is similar to the previous one with a main spacing at 12.2 nm and a minor one at 5.5 nm (small arrow). The low angle background is higher than the two previous cases indicating sample heterogeneity. The molar ratios at which villin and fimbrin were mixed with actin were chosen to give the same molar ratios of these proteins in pellets as found in the microvillar core bundle. The reconstituted specimens lack order although the main interfilament separations are similar.



clearly seen in the Mg^{2+} paracrystals. In the wide angle camera we can detect a series of circular reflections, indexing on about 5.2 nm, out to the sixth order at 0.87 nm. This pattern indicates that the repeat of subunits along either of the actin strands is preserved in the paracrystals and moreover the slight interlocking of the subunits does not destroy the contrast between successive subunits. This is in agreement with oriented F-actin patterns²⁸.

Actin bundled by fimbrin and/or villin: When fimbrin is added to polymerized actin in equimolar amounts, the solution immediately becomes turbid indicating the formation of bundles. The specimens were prepared by centrifugation at 220,000g, which for F-actin alone would produce a spacing of ~11 nm. Similar to the Mg^{2+} paracrystals the fimbrin bundles showed very little orientation, so that in most cases the reflections were smeared out along circles (Fig. 5A). The most prominent reflection is at a Bragg spacing varying between 11.1 and 12.3 nm. Thin sections of bundles fixed before and after centrifugation show that the distance between actin filaments is unaffected. This means that centrifugation only packs bundles, but not actin filaments within bundles. Thus the effect of fimbrin is to cross-link filaments at a distance which otherwise would only be achieved by hard centrifugation. The actin filament substructure is not altered by bundling as the 5.4-nm meridional reflection is observed. In addition, one of four specimens showed a weak 23-nm reflection. Thin sections through fimbrin containing specimens reveal a large amount of aggregated material as well as dispersed actin filaments. This could amount to at most 30% of the total protein, indicating that the spacings of 11–12 nm are produced by the bundles, while the 23-nm reflection could arise from the other aggregates.

Optical diffraction patterns from selected fimbrin bundles show an apparent interfilament spacing of approximately 9.3 nm. This value is considerably larger than that observed with negatively stained Mg^{2+} paracrystals. However, assuming the same degree of shrinkage (25%) as observed with Mg^{2+} paracrystals (see above), the value 9.3 nm agrees well with the X-ray measurements.

When villin is mixed with a solution of F-actin at molar ratios varying between 8:20 and 14:20, one also observes a turbidity increase. The samples were prepared in an identical manner as with fimbrin and the main interparticle interference peak occurred at values between 12 and 13 nm, slightly larger than the case for fimbrin bundles (Fig. 5B). For the reasons given above, the value for the filament separation is governed by villin and not by centrifugation. The slightly larger interfilament separation observed for villin bundles may be related to known differences in native molecular weight between these two proteins (L.K.H. and K.W., unpublished data).

Finally, bundles were assembled as above by adding fimbrin and villin to F-actin, at an initial molar ratio of 20:8:20. This leads to a molar ratio in pelleted bundles of 1:1:10 which is approximately the molar ratio found in the microvillar core

bundles. The scattering pattern is similar to that of the other reconstituted bundles, with the main interparticle interference peak at spacings varying between 12.2 and 12.8 nm. Also present is the meridional 5.4 nm peak (Fig. 5C).

Apart from the variations in spacings, the specimens differ with respect to their order and orientation. For a disoriented specimen with very little order, such as actin bundles (Fig. 5), one observes a disk with a density that seems to terminate abruptly around the first interparticle interference peak. By contrast, specimens with a higher degree of order between filaments show better lattice sampling and the disk of disorder is suppressed relative to the interference peak. This is seen most clearly in the actin patterns (Fig. 4). One conclusion is that although bundling proteins induce actin filaments to assume some degree of interfilament order, it is not as uniform throughout the whole pellet as that observed with actin filaments alone.

Implications for structural roles of bundling proteins

The separation between pure actin filaments can be varied by changing the effective concentration via the centrifugal force. The minimum distance was found to be in the range 12–14 nm, in agreement with earlier results³⁰. Various combinations of fimbrin and villin can induce similar lateral spacings without centrifugation. It seems therefore that the action of bundling proteins is to restrict the diffusion of actin filaments away from one another, rather than to overcome their mutual repulsion which is presumably electrostatic in origin⁴¹. The distances observed with villin were slightly larger than with fimbrin, possibly because of their different molecular sizes.

In isolated microvilli and brush borders we found similar mean distances between actin filaments (12 nm). Their hexagonal arrangement, shown to be present in some thin sectioned microvilli, was confirmed by the presence of a higher order X-ray reflection (1,1) not seen with actin filaments alone. The very low angle region showed clear reflections from the arrangement of microvilli in pellets, indexing approximately on a repeat of 110 nm. This is the first observation of X-ray reflections from an actin-based non-muscle cytoskeleton.

In contrast with fimbrin and/or villin bundles, the separation between actin filaments in Mg^{2+} paracrystals was strongly reduced at approximately 7.0 nm. This 60% decrease suggests that Mg^{2+} acts as an ionic cross-linker, rather than to shield charges. The higher angle reflections compare well with the spacings of actin layer lines measured by Lednev²⁸ from his highly oriented F-actin samples.

The local concentration of actin in microvilli and in reconstituted actin bundles is approximately 3.7 mM, a value approximately 5,000 times higher than concentration for *in vitro* polymerization (~0.03 mg ml⁻¹, or 0.7 μM), and ~15 times higher than the average concentration in the cytoplasm (~10 mg ml⁻¹ or 250 μM). This high concentration of actin is

achieved by the very efficient packing of filaments by fimbrin (0.4 mM) and/or villin (0.6 mM) into a bundle more likely to have a supportive function within the cell than a locomotive one.

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Cloning and expression of human tissue-type plasminogen activator cDNA in *E. coli*

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Bacterial clones containing human tissue-type plasminogen activator (t-PA) cDNA sequences were identified in a cDNA library prepared using gel-fractionated mRNA from human melanoma cells. A plasmid containing the Escherichia coli trp promoter and the cDNA sequence coding for the 527-amino acid mature t-PA protein was constructed for expression in E. coli. A polypeptide was produced having the fibrinolytic properties characteristic of authentic human t-PA.

MAMMALIAN plasma contains an enzymatic system capable of dissolving the fibrin in blood clots. One component of this fibrinolytic system, plasminogen activators, generates the active enzyme plasmin by limited proteolysis of the zymogen plasminogen. Plasmin then degrades the fibrin network of a clot to form soluble products^{1,2}.

There are two drugs commercially available for thrombolytic therapy which function as plasminogen activators³: streptokinase, a bacterial protein, and urokinase, a serine protease isolated from human urine. Thrombolysis with these substances, however, is associated with systemic activation of plasminogen which can produce indiscriminate digestion of coagulation proteins, and significantly increase the risk of haemorrhage during treatment^{4,5}.

Plasminogen activators have been extracted from normal and tumour tissues and are produced by certain cells in culture⁶. The plasminogen activators derived from these sources have been classified into two major groups, urokinase-type plasminogen activators (u-PA) and tissue-type plasminogen activators (t-PA), based on differences in their immunological properties. (The abbreviations t-PA and u-PA are those proposed at the XXVIII Meeting of the International Committee on Thrombosis and Hemostasis, Bergamo, Italy, 27 July 1982.) Recently, a human melanoma cell line has been identified which secretes t-PA. Characterization of this melanoma plasminogen

activator has shown it to be indistinguishable both immunologically and in amino acid composition from the plasminogen activator isolated from normal human tissue⁷.

Several studies *in vitro*⁸⁻¹¹ and *in vivo*¹²⁻¹⁴ which used t-PA purified from the melanoma cell line, have demonstrated its higher affinity for fibrin compared with urokinase-type plasminogen activators. This higher affinity may explain the fact that no systemic activation of plasminogen has been observed to accompany t-PA treatment¹³⁻¹⁵. Preliminary studies in two patients with renal and iliofemoral thrombosis have demonstrated the clinical potential of t-PA as a thrombolytic agent¹⁵.

More extensive investigation of human t-PA as a potential thrombolytic agent has been hampered by its extremely low concentration in blood, tissue extracts, vessel perfusates and cell cultures. The production of t-PA using recombinant DNA techniques should provide sufficient quantities to examine its clinical usefulness in the treatment of pulmonary embolism, deep vein thrombosis, heart attacks and strokes. We report here the isolation and DNA sequence of two recombinant cDNA clones which between them contain the entire coding sequence of human t-PA. We also describe the construction of an expression plasmid which directs the synthesis in *E. coli* of a polypeptide having the fibrinolytic activity of authentic human t-PA.

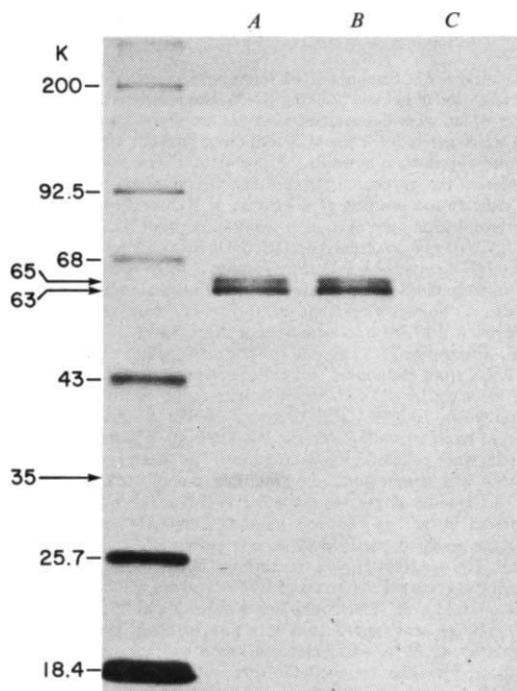


Fig. 1 Immunoprecipitation of melanoma t-PA produced *in vivo*. **A**, t-PA IgG immunoprecipitation; **B**, aprotinin and t-PA IgG immunoprecipitation; **C**, non-immune IgG immunoprecipitation. **Methods:** The Bowes melanoma cell line⁶ was cultured to confluency in a 24-well microtitre dish in Earle's minimal essential medium (EMEM) supplemented with 0.12% sodium bicarbonate, 2 mM glutamine and 10% heat-inactivated fetal calf serum. The cells were washed once with phosphate-buffered saline, and 0.3 ml of serum-free methionine-free EMEM was added to each well. ³⁵S-methionine (75 μ Ci; Amersham) was added and the cells were labelled at 37 °C for 3 h in the presence or absence of 0.33 μ M aprotinin (Trasyol; FBA Pharmaceuticals). After the 3-h labelling period, the medium was removed from the cells and incubated with either t-PA-specific rabbit IgG or non-immune rabbit IgG for immunoprecipitation¹⁷. The immunoprecipitated products were displayed by electrophoresis on a 10% SDS-polyacrylamide gel⁵⁵. The slab gel was fixed, dried and subjected to fluorography⁵⁶. Antisera against tissue-type plasminogen activator were raised in rabbits by multiple-site intradermal injections of 100 μ g of purified protein dissolved in 0.5 ml of saline and emulsified with 0.5 ml of complete Freund's adjuvant. Booster injections of 50 μ g were given at 2-week intervals and serum was collected after the second or third booster. The IgG fractions of the antisera were isolated by affinity chromatography on protein A-Sepharose and dialysed against 0.15 M NaCl solution.

Table 1 Plasminogen activation by extracts of *E. coli*

<i>E. coli</i> W3110 transformed by	Treatment	ΔA_{405}	Units per A_{550} cells
pt-PATrp12	—	0.657	2.9
pt-PATrp12	Non-immune IgG	0.665	2.9
pt-PATrp12	t-PA IgG	0.059	0.26
pLeIFATrp103	—	0.055	0.25

E. coli cultures were grown and extracts prepared as described in Fig. 5 legend. A 20- μ l aliquot of each extract was incubated at 37 °C for 10 min with 0.15 ml of a 50 mM Tris-HCl pH 7.4, 12 mM NaCl solution containing 0.37 mg ml⁻¹ plasminogen and 2.6 mg ml⁻¹ fibrinogen. 0.35 ml of a 1 mM solution of S2251 (Kabi) in the above buffer was added and the reaction continued for 5–30 min at 37 °C. Acetic acid was added to a final concentration of 0.8 M to stop the reaction. The samples were centrifuged and absorbance at 405 nm measured. Where indicated, ~30 μ g of either rabbit non-immune IgG or t-PA-specific IgG was added to the initial incubation. Units were defined by comparison to a urokinase standard (Calbiochem). The published specific activity of pure t-PA is ~60,300 PU mg⁻¹ (refs 7, 46).

Immunological characterization of t-PA and mRNA isolation

To characterize the plasminogen activator species secreted from the Bowes human melanoma cell line⁷, confluent monolayers of the cells were grown and the proteins labelled for 3 h with ³⁵S-methionine. The cell medium was immunoprecipitated with t-PA-specific rabbit IgG and electrophoresed on a SDS-polyacrylamide gel. Three different forms of melanoma t-PA were observed having molecular weights (MWs) of ~65,000 (65K), 63K and 35K (Fig. 1A). These three species correspond to those observed following electrophoresis and Coomassie blue staining of purified melanoma t-PA (data not shown). When the protease inhibitor, aprotinin, was added during the 3-h labelling period, only the 65K and 63K MW species were observed (Fig. 1B). Aprotinin has been shown previously to prevent the processing of single-chain t-PA to the two-chain form⁷. No immunoprecipitated polypeptides were observed when non-immune rabbit IgG was substituted for t-PA-specific IgG (Fig. 1C).

To determine whether the 65K and 63K MW species are related forms of t-PA, limited peptide mapping was performed using staphylococcal V8 protease^{16,17}. Analysis of the peptides on SDS-polyacrylamide gels revealed that the two species are closely related in peptide composition, one probably being a derivative of the other (data not shown). The molecular weight difference might be due to a variation in carbohydrate composition or to the presence of an additional peptide in the 65K species. However, on sequence analysis both forms were found to have the same N-terminal amino acid sequence (W.J.K. and R.N.H., unpublished results).

The Bowes melanoma cell line was used as the source of RNA for cDNA cloning experiments. Polyadenylated RNA was prepared¹⁸ and fractionated by electrophoresis through urea-agarose gels^{19–21} to enrich for t-PA mRNA. Aliquots of the individual mRNA fractions were assayed for the presence of t-PA mRNA by *in vitro* translation in a rabbit reticulocyte lysate system²² supplemented with dog pancreas microsomes²³. The resulting translation products were immunoprecipitated with t-PA-specific IgG and analysed by SDS-polyacrylamide gel electrophoresis (Fig. 2A). One major polypeptide having ~63,000 MW was observed for both RNA fractions 7 and 8. This band was not observed when non-immune rabbit IgG was used for immunoprecipitation (Fig. 2B, lanes a and d) of either total RNA or gel slice 7 RNA translation products, which suggested that the polypeptide was t-PA. The size of the mRNA in fractions 7 and 8 is ~21–24S (2,400–2,700 nucleotides).

Construction and identification of bacterial clones containing t-PA cDNA sequences

RNA from gel slice 7 (Fig. 2A) was used to prepare double-stranded cDNA by standard procedures^{24,25}. The cDNA was fractionated by size, and material longer than 350 base pairs (bp) was extended with deoxy(C) residues, annealed to deoxy(G)-tailed *Pst*I-cleaved pBR322, and used to transform *E. coli* K-12 strain 294 (ref. 26) as previously described²⁵; ~4,600 transformants were obtained (see legend to Fig. 2B).

To prepare a specific DNA hybridization probe, we determined the amino acid sequences of several tryptic fragments of t-PA purified from melanoma cells (W.J.K. and R.N.H., unpublished results). This information permitted the design of synthetic deoxynucleotides potentially complementary to a specific region of t-PA mRNA. The amino acid sequence (Trp-Glu-Tyr-Cys-Asp) of a region of a purified tryptic peptide was selected for preparation of a probe because it required the synthesis²⁷ of only eight tetradecanucleotides (dTC₆CA₆TA₆TCCCA) to account for all possible anti-coding sequences. This pool of 14-mers was labelled with ³²P and used to screen the 4,600 cDNA clones by *in situ* colony hybridization²⁸. Plasmid DNA was isolated²⁹ from 12 colonies that gave a positive hybridization signal. DNA sequencing was performed on the cDNA inserts from each of these clones by the dideoxy

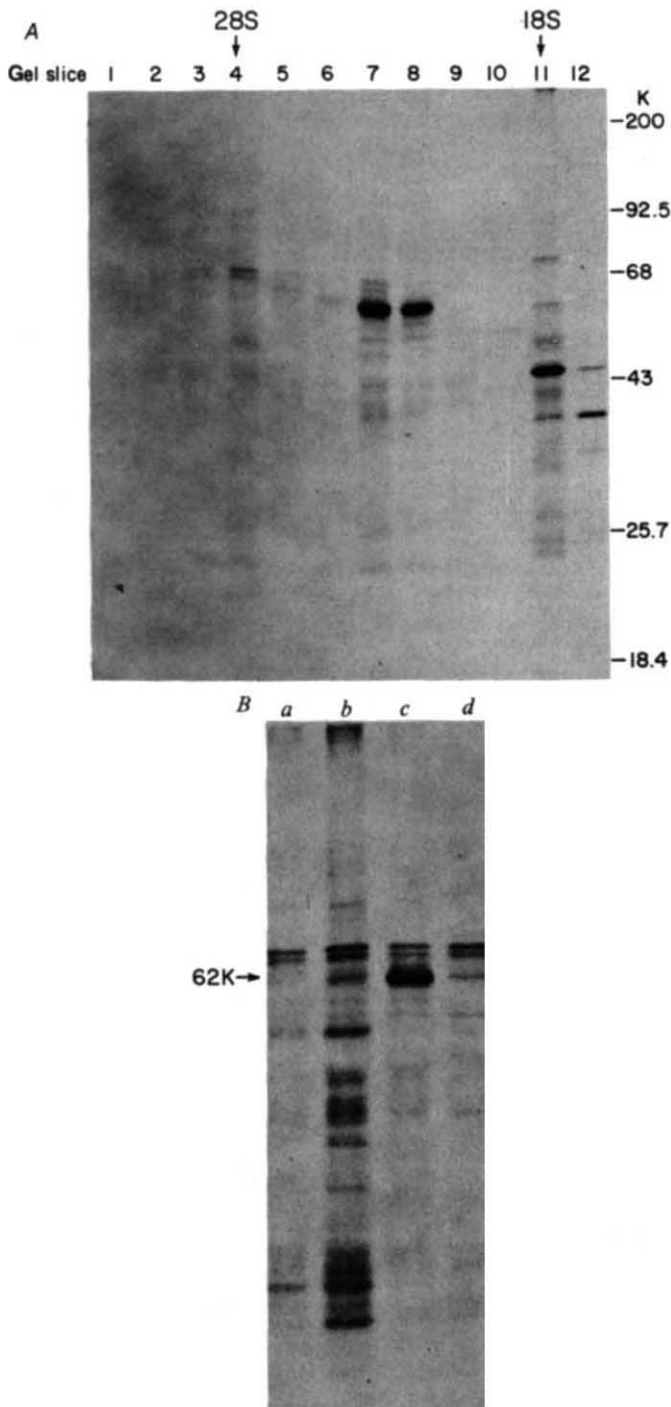


Fig. 2 *A*, gel electrophoresis of immunoprecipitated products from *in vitro* translation of gel-fractionated RNA. The positions of ribosomal RNA markers which were determined after electrophoresis in an adjacent lane on the RNA gel and visualized by ethidium bromide staining are labelled above the appropriate lanes of the protein gel. The numbers above each lane refer to the particular gel slice and the numbers to the right of the figure indicate the position of migration of ^{14}C -labelled protein markers. *B*, immunoprecipitation of *in vitro* translation products using non-immune rabbit IgG. Total RNA translation products immunoprecipitated with non-immune IgG (*a*) or t-PA IgG (*b*). Gel slice 7 mRNA translation products immunoprecipitated with t-PA IgG (*c*) or non-immune IgG (*d*).

Methods: *A*, human melanoma cells (Bowes) were grown to confluent monolayers in EMEM in plastic tissue culture flasks (175 cm²; style 3028, Falcon Labware) at 37 °C in atmospheric air supplemented with 6% CO₂. Total RNA from melanoma cell cultures was extracted essentially as reported by Ward *et al.*²⁷. Cells were pelleted by centrifugation and then resuspended in 10 mM NaCl, 10 mM Tris-HCl pH 7.5, 1.5 mM MgCl₂. Cells were lysed by adding Nonidet P-40 (NP-40; 1% final concentration), and nuclei were pelleted by centrifugation. The supernatant containing the total RNA was further purified by multiple phenol and chloroform extractions. The aqueous phase was made 0.2 M in NaCl and the total RNA was precipitated by adding two vols ethanol. Oligo(dT)-cellulose chromatography was used to purify poly(A)-containing RNA¹⁷. Fractionation of poly(A)⁺ RNA (200 µg) was performed by electrophoresis through a denaturing agarose gel composed of 1.75% agarose, 0.025 M sodium citrate pH 3.8 and 6 M urea. Electrophoresis was for 7 h at 25 mA and 4 °C (refs 19–21). The gel was fractionated with a razor blade and individual slices were melted at 70 °C and extracted twice with phenol and once with chloroform. Fractions were ethanol-precipitated and then assayed by *in vitro* translation in a rabbit reticulocyte lysate system²² supplemented with dog pancreas microsomes²³. Incubations were at 30 °C for 90 min. The resulting translation products from each gel fraction were immunoprecipitated with rabbit IgG directed against human t-PA and analysed by electrophoresis on 10% SDS-polyacrylamide gels. The gels were fixed, dried and fluorographed²⁶. *B*, 11 µg of total melanoma RNA or ~340 ng of gel slice 7 poly(A)⁺ RNA were translated in a rabbit reticulocyte lysate system²² supplemented with dog pancreas microsomes²³; 11 µg of total RNA contains between 330 and 550 ng of mRNA based on the finding that ~3–5% of total RNA is mRNA⁵⁸. The resulting translation products were immunoprecipitated with either rabbit IgG directed against human t-PA or non-immune rabbit IgG and analysed by electrophoresis on 10% SDS-polyacrylamide gels. Double-stranded cDNA was prepared by standard methods^{24,25,40} using 5 µg of mRNA from gel fraction 7. The cDNA longer than 350 bp (125 ng) was recovered by electroelution²⁵ after fractionation on a 6% polyacrylamide gel, 30 ng of cDNA was extended with deoxy(C) residues using terminal deoxynucleotidyl transferase⁵⁹ and annealed with 300 ng of the plasmid pBR322⁶⁰ which had been similarly tailed with deoxy(G) residues at the *Pst*I site. The annealed mixture was used to transform *E. coli* K-12 strain 294 (ref. 25) and resultant tetracycline-resistant colonies were individually inoculated into wells of microtitre plates containing L broth⁶¹ and 5 µg ml⁻¹ tetracycline. The cDNA library of 4,600 transformants was grown up on nitrocellulose filters and the DNA from each colony was fixed to the filter²⁸. The eight deoxyoligonucleotides (dTCA⁺CA⁺TA⁺TCCCA) were chemically synthesized in two pools of four 14-mers by the solid-phase phosphotriester method²⁷. A ^{32}P -labelled probe was prepared from the pool of eight 14-mers as described previously⁴⁰. The set of filters containing the 4,600 transformants was hybridized to 5 × 10⁷ c.p.m. of the labelled probe in 50 mM sodium phosphate pH 6.8, 5 × SSC, 150 µg ml⁻¹ sonicated salmon sperm DNA, 5 × Denhardt's solution and 10% formamide. After 16 h at room temperature, the filters were washed extensively at room temperature in 6 × SSC and 0.1% SDS, then exposed to X-ray film.

chain termination procedure³⁰ after subcloning fragments into the M13 vector mp7 (ref. 31), or by the Maxam-Gilbert chemical procedure³². Only one cDNA insert, that of colony 25E10, contained sequences which could code for the amino acid sequence of the tryptic peptides of melanoma t-PA (W.J.K. and R.N.H., unpublished results). The cDNA insert of plasmid pPA25E10 was sequenced and found to be 2,304 bp long. The sequence (beginning with nucleotide 243 of Fig. 3B) contains an open reading frame encoding a protein of 508 amino acids and a 745-bp 3' untranslated region.

Preparation of a colony library containing NH₂-terminal t-PA sequences

The cDNA clone pPA25E10 was not a full-length copy of t-PA mRNA as it lacked the t-PA NH₂-terminal coding sequences determined by amino acid sequencing of the purified protein. Therefore, it was necessary to produce cDNA clones containing the 5' portion of the t-PA mRNA. A 16-base long deoxy-

oligonucleotide complementary to nucleotides 256–271 of the t-PA mRNA and having the sequence dTTCTGAGCACAGGGCG was synthesized²⁷. This hexadecanucleotide was used to prime cDNA synthesis³³ of fraction 7 mRNA; 1,500 clones were obtained. As the cDNA priming reaction was performed using a synthetic fragment that hybridized only 13 bp from the 5' terminus of the pPA25E10 cDNA, no convenient restriction fragment was available for use as a probe to screen for t-PA cDNA clones containing t-PA sequences 5' of those in pPA25E10. We therefore isolated a genomic clone for t-PA from a human gene library³⁴ and used this as a hybridization probe to identify primer-extended cDNA clones containing NH₂-terminal t-PA coding sequences.

The first step in this process was to determine whether only a single homologous t-PA gene was present in human genomic DNA. Southern hybridizations³⁵ were performed using high-molecular weight human DNA which had been digested with various restriction endonucleases. The ^{32}P -labelled³⁶ cDNA

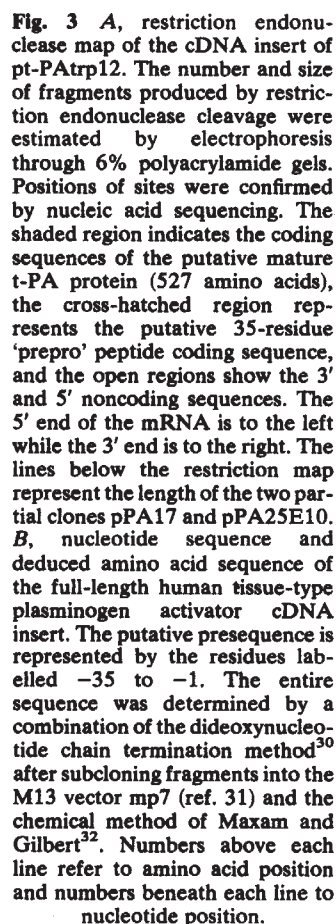


Fig. 4 Construction of a plasmid coding for the direct expression of mature t-PA in *E. coli*. 50 µg of plasmid pPA17 was digested with *Sau*3AI, *Hinc*II and *Hha*I and electrophoresed on a 6% polyacrylamide gel; ~0.5 µg of the 55-bp *Sau*3AI-*Hha*I fragment was recovered. Similarly, ~3 µg of the 263-bp *Hha*I-*Nar*I fragment was purified from 80 µg of clone pPA25E10 by first isolating a 300-bp *Pst*I-*Nar*I fragment and then digesting this fragment with *Hha*I. All digests were performed at 37 °C for 1 h and the reaction products resolved and electroeluted from 6% polyacrylamide gels. The two indicated deoxyoligonucleotides 5'-dAATTCATGTCTTATCAAGT (I) and 5'-GATCACTTGATAAGACATG (II) were synthesized by the solid-phase phosphotriester method²⁷. 100 pmol of oligonucleotide II was phosphorylated in 30-µl reaction mixture containing 60 mM Tris pH 8, 10 mM MgCl₂, 15 mM β-mercaptoethanol and 50 µCi [γ -³²P]ATP (5,000 Ci mmol⁻¹; Amersham). T4 polynucleotide kinase (12 units) was added and the reaction allowed to proceed at 37 °C for 15 min; 1 µl of 10 mM ATP and 12 units of T4 kinase were then added and the reaction allowed to proceed for an additional 30 min. After phenol and chloroform extraction, the phosphorylated oligomer II and the 5'-hydroxyl oligomer I were combined with 0.5 µg of the eluted 55-bp *Sau*3AI-*Hha*I fragment and 2 µg of the 263-bp *Hha*I-*Nar*I fragment and ethanol-precipitated. These fragments were ligated at room temperature for 4 h in 60 µl of 20 mM Tris-HCl pH 7.5, 10 mM MgCl₂, 10 mM dithiothreitol, 0.5 mM ATP and 1,000 units of T4 DNA ligase. The mixture was digested for 1 h with 48 units of *Nar*I, 20 units of *Eco*RI and 40 units of *Bgl*II (to eliminate polymerization through ligation of cohesive *Sau*3AI termini) and electrophoresed on a 6% gel. The 338-bp product (~0.1 µg; 1,385 c.p.m.) was recovered by electroelution. The remainder of the t-PA-coding sequences (amino acids 111-528) were isolated on a 1,645-bp fragment by digesting plasmid pPA25E10 with *Nar*I and *Bgl*II. The plasmid pLeIFAtpr103 is a derivative of the plasmid pLeIFAt25 (ref. 25) in which the *Eco*RI site distal to the LeIF A gene has been removed¹⁹. pLeIFAtpr103 (3 µg) was digested with 20 units of *Eco*RI and 20 units of *Bgl*II for 90 min at 37 °C, electrophoresed on a 6% polyacrylamide gel and the large (~4,200 bp) vector fragment was recovered by electroelution. For the final construction, 80 ng of *Eco*RI-*Bgl*II pLeIFAtpr103 was ligated with 100 ng of the 1,645-bp *Nar*I-*Bgl*II fragment and 20 ng of the 338-bp *Eco*RI-*Nar*I fragment at room temperature for 10 h. This ligation mixture was used to transform *E. coli* K-12 strain 294 (ref. 26). Plasmid DNA was prepared from 38 of the transformants and digested with *Eco*RI; 10 of these plasmids contained the desired 600- and 472-bp *Eco*RI fragments. DNA sequence analysis verified that one of these plasmids (pt-PAtpr12) had the desired nucleotide sequence at the junctions between the *trp* promoter, synthetic DNA and cDNA.

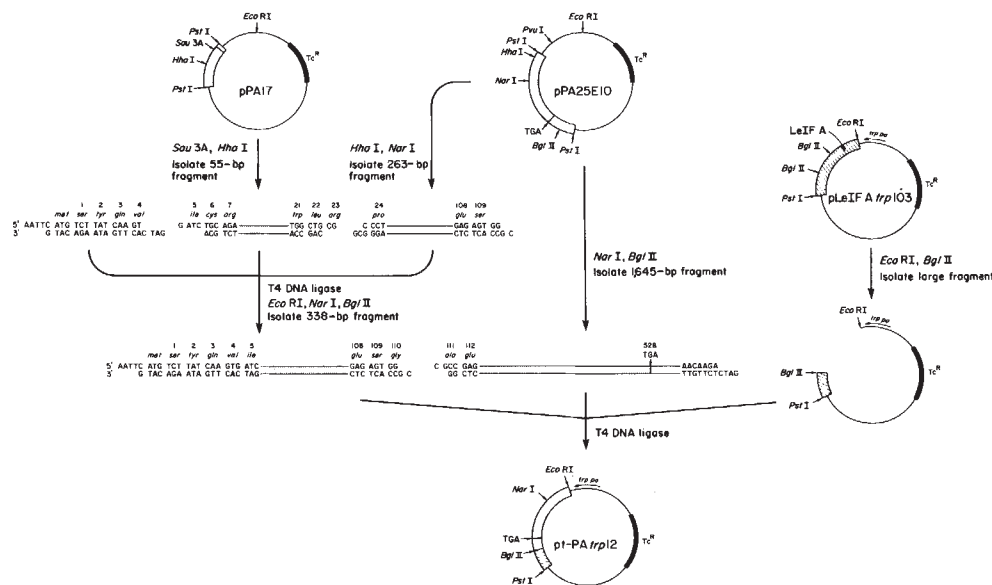
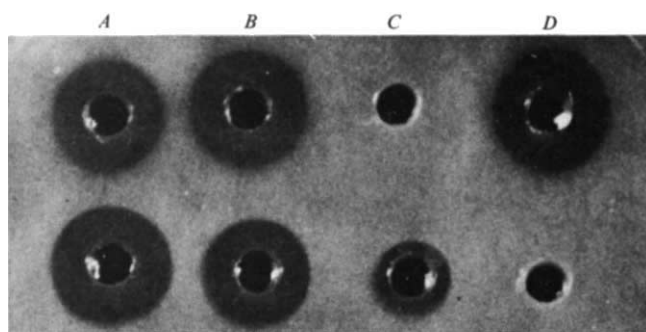


Fig. 5 Characterization of t-PA produced by *E. coli*. Wells A-D contain 10 µl of a pt-PAtpr12 cell extract plus the following additions: A, none; B, non-immune IgG; C, t-PA IgG; D, urokinase IgG. Wells E-G contain, respectively, 0.2, 0.1 and 0.02 unit purified melanoma t-PA. Well H contains 10 µl of an extract from cells containing the plasmid pLeIFAtpr103.

Methods: An overnight culture of *E. coli* W3110/pt-PAtpr12 in Luria broth⁵⁷ containing 5 µg ml⁻¹ tetracycline was diluted 1:100 in M9 medium⁵⁷ containing 0.2% glucose, 0.5% casamino acids and 5 µg ml⁻¹ tetracycline. The cells were grown at 37 °C to A₅₅₀ of 0.2 and indole acrylic acid was added to a final concentration of 20 µg ml⁻¹. Samples were collected, by centrifugation, at A₅₅₀ = 0.5-0.6 (~2 × 10⁸ cells ml⁻¹) and immediately frozen. The cell pellets were suspended in 6 M guanidine hydrochloride at 5 × 10⁸ cells ml⁻¹, sonicated for 10 s, incubated at 24 °C for 30 min and then dialysed (buffer flow rate 250 ml h⁻¹) for 4 h against 50 mM Tris-HCl pH 8.0, 250 mM NaCl, 0.25 mM EDTA and 0.01% Tween 80, using a continuous flow microdialysis system (Bethesda Research Labs). After dialysis the samples were centrifuged at 13,000g for 2 min and 10-µl samples of the supernatants (1 ml) analysed for t-PA activity. Following the procedure of Granelli-Piperno and Reich⁴⁵, the plate was incubated for 3.5 h at 37 °C and lysis zones measured. Activity was approximated by comparison with dilutions of a standard purified melanoma t-PA solution.



probe used was a 232-bp *Rsa*I-*Pst*I fragment (nucleotides 388-620 of Fig. 3B) prepared from the 5' end of the cDNA insert of clone pPA25E10. Two endonuclease digestion patterns provided only a single hybridizing DNA fragment: *Bgl*II (5.7 kilobase pairs, kbp) and *Pvu*II (4.2 kbp). Two hybridizing DNA fragments were observed with *Hinc*II (5.1 and 4.3 kbp) (data not shown). Comparison of these data with the cDNA restriction map (Fig. 3A) suggests that there is only one t-PA gene in the human genome and that this gene contains at least one intervening sequence.

Approximately 10⁶ plaques of a λ Charon 4A/human genomic library³⁴ were screened³⁷ with the ³²P-labelled 232-bp

*Rsa*I-*Pst*I fragment; 19 individual clones were isolated and phage DNA was prepared³⁸. A 4.2-kbp *Pvu*II fragment containing t-PA sequences was isolated from one of these clones, labelled with ³²P and used to screen the 1,500 clones of the 5' primer-extended cDNA library. Plasmid DNAs were prepared from the 18 colonies which gave positive hybridization signals and these DNAs were bound to a nitrocellulose filter.^{39,40}

To identify clones having cDNA inserts which overlapped with the cDNA insert of pPA25E10, the filter was hybridized with the ³²P-labelled synthetic oligonucleotide (16-mer) used for the original priming reaction. Of the 18 selected cDNA clones, 7 hybridized with the ³²P-labelled 16-mer; however, on

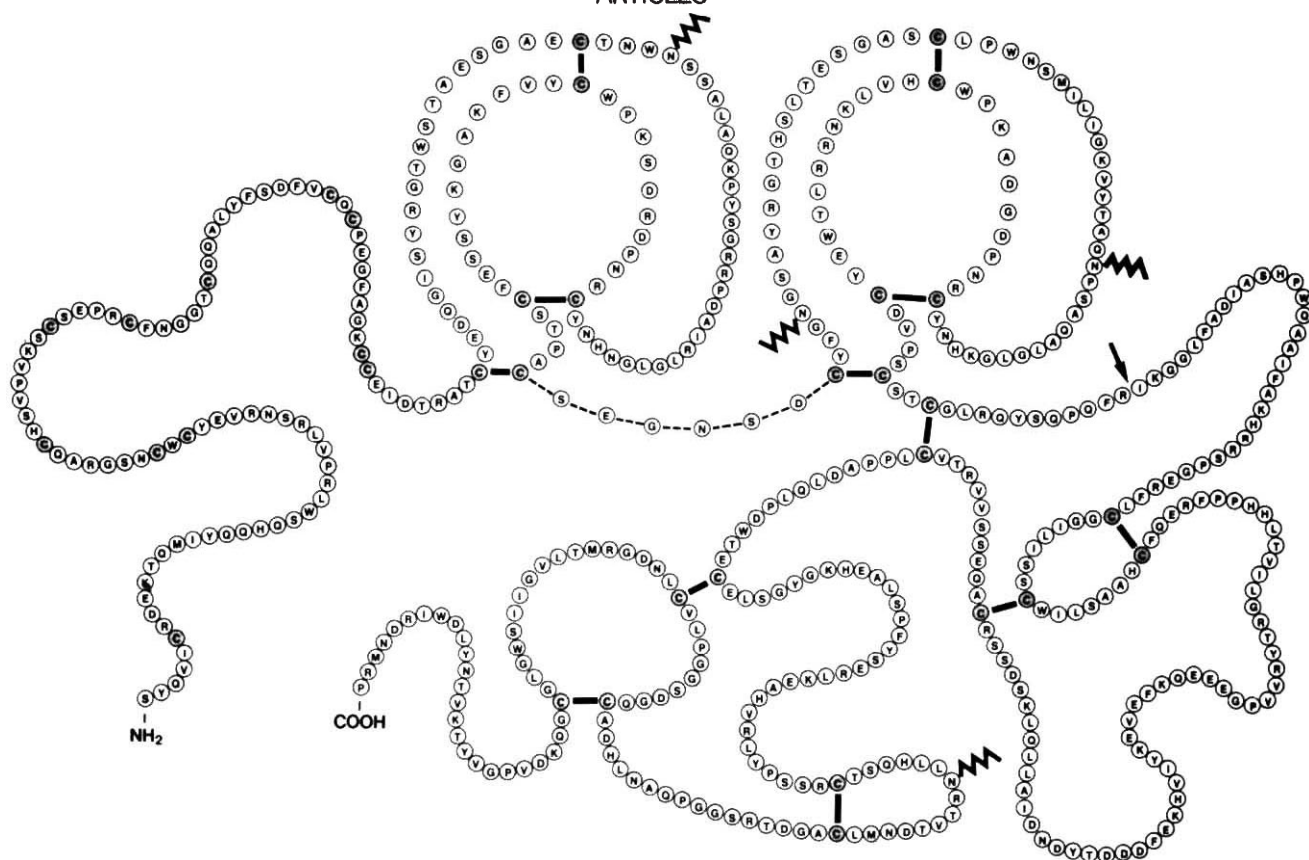


Fig. 6 Schematic diagram of the potential structure of human tissue-type plasminogen activator. The one-letter code for each of the amino acids is given in the open circles. The one-letter abbreviations are those recommended by the IUPAC-IUB Commission on Biochemical Nomenclature⁵⁴. The cysteine residues are shaded. The solid black bars indicate the potential disulphide bridges based on homology with other serine proteases. The arrow indicates the potential cleavage site between arginine and the isoleucine which generates the two-chain molecule from the one-chain form. The zig-zag lines indicate potential glycosylation sites. The broken lines connect the six amino acids between the two kringles.

sequence analysis only one of the cDNA clones, pPA17, was found to contain the complete NH₂-terminal region of t-PA and to overlap with the original clone, 25E10.

The cDNA insert of pPA17 is 271 bp long. It contains the hexadecanucleotide sequence used to prime its synthesis, which permitted the alignment of its DNA sequence with that of pPA25E10. From these two cDNA clones, pPA15E10 and pPA17, we determined the nucleotide sequence and corresponding amino acid sequence of t-PA (Fig. 3B).

The complete 2,530-bp cDNA sequence contains a single open reading frame, beginning with the ATG codon at nucleotides 85–87. This ATG is followed, 562 codons later, by a TGA termination triplet at nucleotides 1,771–1,773. This ATG probably serves as the site of translation initiation as it is the first one encountered and it is preceded in phase at nucleotides 4–6 by a termination codon. The serine designated as amino acid number 1 is based on NH₂-terminal sequencing of purified melanoma cell t-PA. This serine is preceded by 35 amino acids, the NH₂-terminal 20–23 of which probably constitute a hydrophobic signal peptide⁴¹ involved in the secretion of t-PA. The remaining 12–15 hydrophilic amino acids immediately preceding the start of mature t-PA may constitute a 'pro' sequence similar to that found for serum albumin^{33,42}. The 3' untranslated region of 759 nucleotides contains the hexanucleotide AATAAA (position 2,496–2,501) which precedes the site of polyadenylation in many eukaryotic mRNAs⁴³. The native t-PA molecule has 35 cysteine residues and thus has the potential to be stabilized by 17 disulphide bridges. There are four potential N-glycosylation sites⁴⁴, located at Asn 117, Asn 184, Asn 218 and Asn 448 in t-PA. We have confirmed only the carbohydrate on Asn 448 in native t-PA (W.J.K. and R.N.H., unpublished results). Variations in the structure of the oligosaccharide

ligands may account for the different molecular forms of t-PA (65K and 63K species) observed.

Synthesis of tissue-type plasminogen activator in *E. coli*

The procedure followed to directly express the full-length cDNA insert of t-PA in *E. coli* is outlined in Fig. 4. The common *HhaI* restriction endonuclease site shared by the cDNA inserts of both partial clones pPA17 and pPA25E10 permitted the reconstruction of the entire mature t-PA coding sequence. A 55-bp *Sau3AI-HhaI* restriction fragment corresponding to amino acids 5–23 was isolated from the plasmid pPA17. The *Sau3AI* restriction site is located at codon 4 of the presumed mature coding sequence and was used to remove the 'prepro' peptide-coding region. A 263-bp *HhaI-NarI* fragment (encoding amino acids 14–110) was isolated from plasmid pPA25E10. Two synthetic deoxyoligonucleotides were designed to restore the codons for amino acids 1–4, incorporate an ATG translational initiation codon and create an *EcoRI* cohesive terminus. These fragments were then ligated together to form a 338-bp fragment coding for amino acids 1–110 of t-PA. This fragment and a 1,645-bp *NarI-BglII* fragment from pPA15E10 were then ligated between the *EcoRI* and *BglII* sites of the plasmid pLeIFAt_{trp}103 (ref. 21) to give the expression plasmid, pt-PA_{trp}12. The cloned t-PA gene is transcribed under the control of a 300-bp fragment of the *E. coli trp* operon which contains the *trp* promoter, operator and the Shine-Dalgarno sequence of the *trp* leader peptide, but lacks the leader peptide ATG initiation codon⁴⁰.

E. coli K-12 strain W3110 (ATCC no. 27325) containing the plasmid pt-PA_{trp}12, was grown, and extracts prepared for assay of fibrinolytic activity using the fibrin plate technique⁴⁵.

This assay involves the use of an agarose plate containing plasminogen and fibrin; when a sample containing a plasminogen activator is added to a well in the agarose, the plasminogen is converted to the active enzyme plasmin. The plasmin so formed then degrades the fibrin to give a clear zone of lysis surrounding the well. The area of this lysis zone can be correlated to the amount of plasminogen activator in the sample. Figure 5 shows that when extracts from pt-PATrp12 clones were tested for plasminogen activator activity using this assay, a clear zone of lysis was evident (well A). This fibrinolytic activity was inhibited by t-PA-specific IgG (well C) but not by non-immune IgG (well B) or urokinase-specific IgG (well D) and no activity was seen in an extract prepared from cells containing as a control the α -interferon expression plasmid pLeIFATrp103 (well H). Using the standard curve of purified melanoma cell t-PA (wells E–G) we estimated that ~5 units of t-PA activity could be extracted per A_{550} of culture.

t-PA activity can also be measured using a chromogenic substrate assay. This assay measures the rate of conversion of plasminogen to plasmin by t-PA. The amount of plasmin formed is determined by monitoring the proteolytic cleavage of a tripeptide from the chromogenic substrate, S2251. Table 1 shows the results obtained with this assay for *E. coli* W3110/pt-PATrp12 extracts. The activity (~3 units per A_{550} of culture) measured by this assay is also neutralized by t-PA-specific IgG but not by non-immune IgG. The published specific activity of melanoma t-PA is 60,300 Plough units (PU) mg^{-1} (refs 7, 46).

Conclusion

Human melanoma cells actively synthesizing and secreting t-PA were used for the preparation of poly(A) mRNA. This mRNA was size-fractionated using agarose gel electrophoresis and the fractions containing t-PA mRNA were used to prepare a cDNA library, which was hybridized with a pool of radiolabelled synthetic deoxynucleotides potentially complementary to mRNA encoding a known amino acid sequence of t-PA. A recombinant plasmid (pPA25E10) containing a partial-length t-PA cDNA insert was identified and sequenced. The remaining 5' cDNA sequences were generated by preparing a cDNA library primed with a 16-base long synthetic fragment. A t-PA cDNA clone (pPA17) overlapping with the original clone pPA25E10 and containing the NH_2 -terminal and presequence regions was identified after specific hybridization with a DNA fragment prepared from a t-PA genomic clone.

The 2,530 bp of t-PA cDNA (excluding the poly(A) sequence) code for a polypeptide of 562 amino acids. The 35 amino acids (–35 to –1) preceding the mature sequence probably constitute a 20–23-amino acid long hydrophobic signal peptide followed by a hydrophilic 'pro' sequence of 12–15 amino acids. This type of prepro structure on secreted proteins has been described previously, for example, with prealbumin^{33,42}. On this assumption, all the secreted one-chain t-PA molecules will have a serine residue at the amino terminus. An alternative possibility is that the hydrophilic 'pro' sequence could be removed in a manner analogous to that observed with plasminogen where a peptide of MW 10,000 can be cleaved from the amino-terminal portion of native plasminogen (Glu-plasminogen, named for the amino terminal residue), resulting in a protein having a new amino terminus, designated Lys-plasminogen^{47,48}.

It has been demonstrated that two-chain t-PA is formed by proteolytic cleavage of the single-chain molecule into two polypeptides connected by disulphide bonds⁷. NH_2 -terminal sequencing of the two-chain molecule suggests that it is generated by cleavage of the Arg 275–Ile 276 peptide bond (W.J.K. and R.N.H., unpublished results) (see Fig. 6). As determined from the primary structure, the heavy chain (MW 30,882) is derived from the NH_2 -terminal part and the light chain (MW 28,126) comprises the COOH-terminal region (Fig. 6).

The primary structure of a portion of the heavy-chain region of t-PA has a high degree of sequence homology with the 'kringle' regions of plasminogen⁴⁹ and prothrombin^{50,51}

'Kringle' refers to a characteristic triple disulphide structure originally discovered in the 'pro' fragment of prothrombin and described in detail by Magnusson *et al.*^{51,52}. From the primary sequence of t-PA, two such regions of 82 amino acids each (residues 92–173 and 180–261; Fig. 6) are apparent that share a high degree of homology with the five kringles of plasminogen. We have also recently determined that human urinary urokinase contains one similar triple disulphide region⁵³. The remaining NH_2 -terminal 91 amino acids of t-PA share little homology to the conventional kringle region. One can speculate, however, that this region may also assume a structure containing multiple disulphide bonds as 11 additional cysteine residues are found here.

The catalytic site of the light chain of t-PA, as in other serine proteases, is probably formed by the His 322, Asp 371 and Ser 478 residues. Furthermore, the amino acid sequences surrounding these residues are highly homologous to corresponding parts of other serine proteases such as trypsin, prothrombin and plasminogen⁵⁴.

The amounts of t-PA activity (3–5 units per A_{550} , depending on the assay used) recovered from the *E. coli* extracts correspond to ~50–80 μg per l of culture at $A_{550} = 1$, or ~1,500–2,400 fully active t-PA molecules per cell. This activity has been shown to activate plasminogen catalytically and is neutralized by t-PA-specific IgG antibody. As only functional activity was measured, it is possible that considerably more t-PA is present in these cells. In addition, because polypeptides synthesized in *E. coli* are not glycosylated, the t-PA synthesized by *E. coli* might have a specific activity different from that of authentic t-PA.

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LETTERS TO NATURE

A QSO in a rich, distant cluster of galaxies

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We have previously remarked¹ that there are no confirmed cases of quasistellar objects (QSOs) that are members of rich clusters of galaxies. Several candidates exist (cited in ref. 1), but all suffer from defects. In some of these cases, the linear dimensions may be more appropriate for superclusters than clusters (an interesting result nonetheless), and in other cases only the QSO members of the putative clusters are visible, and the normal galaxies are inferred but not observed. A particularly direct confirmation that QSOs share the same clustering characteristics as other constituents of the Universe would come from the detection of a 'classical' (for example, point-like, high luminosity) QSO in a previously studied, distant rich cluster of visible galaxies. Here we report such a discovery.

The cluster 0016+16, at $z = 0.541$ (ref. 2) is perhaps the best studied distant cluster of galaxies. Detailed multicolour photometry and isopleths³ show this cluster to be richer than the Coma Cluster, and remarkably normal except for its very large redshift. The brightest members have red magnitudes of ~ 20.4 . The cluster is a luminous, extended X-ray source⁴, indicating the presence of an ionized intracluster gas as is common in rich clusters. There is also a detection⁵ of a decrement in the microwave background radiation against 0016+16, so the intracluster gas parameters can be studied in some detail, and (at least in principle) the cosmological parameters q_0 and H_0 may be estimated⁶.

During X-ray observations of 0016+16, a previously unreported point source near the northern edge of the visible galaxy distribution was detected⁴, and the identification of this source with an unstudied $B \sim 18$ blue stellar object has been suggested^{3,4}. We have found^{7,8} that a large fraction of these serendipitously-observed faint X-ray sources are associated with previously uncatalogued QSOs. With this motivation, we obtained medium resolution ($10 \text{ \AA}$) spectroscopy of the 18th mag object. On 25 July 1982, the blue spectrum was observed with the SIT Vidicon spectrometer⁹ at the 4-m reflector of the Cerro Tololo Interamerican Observatory; these data were converted to absolute fluxes through observation of spec-

trophotometric standard stars. The red/IR region of the spectrum was observed on 15 October 1982, using the image tube scanner¹⁰ at the Lick Observatory 3-m Shane telescope.

The resulting spectra are shown in Fig. 1. This object is clearly a QSO, which we denote as 0015+162. We identify the prominent broad emission lines at 4,347, 6,749 and 7,557 Å as Mg II $\lambda 2,800$, H γ and H β , respectively, and derive redshift $z = 0.554 \pm 0.002$. There is a marginally positive detection of [O III] $\lambda 5,007$ emission, at an atypically weak intensity, less than H β . The [O III] $\lambda 4,959$ line is not separable from the very strong OH $\lambda 7,715$ night-sky emission at our spectral resolution, and similarly any [O II] $\lambda 3,727$ emission would coincide very closely with the Hg $\lambda 5,790$ emission from city lights, which appears very strongly in the Lick data. Our spectrophotometry yields $B \sim 18.2$, $(B - V) \sim 0$, which for $q_0 = 0$, $H_0 = 50 \text{ km s}^{-1} \text{ Mpc}^{-1}$ implies an absolute V magnitude of -25 . Our magnitude combined with the observed X-ray intensity⁴ implies a value for α_{ox} , the slope of a line connecting the optical and X-ray fluxes, of 1.32. This is quite close to the mean found for both serendipitously-discovered^{7,8} and previously known^{11,12} QSOs, and suggests that 0015+162 is quite a normal quasar. Previous radio observations⁵ searching for point sources near the cluster indicate the object to be radio-quiet, as is expected for a randomly-selected QSO.

There seems little doubt that the QSO 0015+162 and cluster 0016+16 are related. The surface density of QSOs this bright is very small¹³. We expect less than one QSO this bright to occur by accident at any redshift and at any location on the entire 4-m telescope plate that discovered the cluster, much less at a redshift essentially identical to that of the cluster, co-located on the 10^{-2} of the plate that contains the cluster. The simplest explanation of this association is that the QSO 0015+162 is a member of the rich cluster of galaxies 0016+16. The formal velocity difference between the objects is currently $2,000 \text{ km s}^{-1}$ in the observed frame, which must contain components due to the observational uncertainty in our determination of the QSO redshift, the analogous quantity for the exceedingly faint galaxy observed to obtain the cluster redshift, and the velocity dispersion expected for a rich cluster. Each of these three individual quantities, much less their combination, is of magnitude comparable with the observed difference. The core radius of the cluster³ is measured as 0.75 arc min ($\sim 0.4 \text{ Mpc}$), implying that the QSO is 4.5 core radii from the optical and X-ray centre of the cluster. Such an offset is, of course, quite consistent with cluster membership. In the classic study of membership of the Coma Cluster¹⁴, for example, 60% of all galaxies assigned as cluster members are more distant than 4 core radii from the cluster centre. A more recent study¹⁵ finds an even more extreme percentage of members beyond this distance.

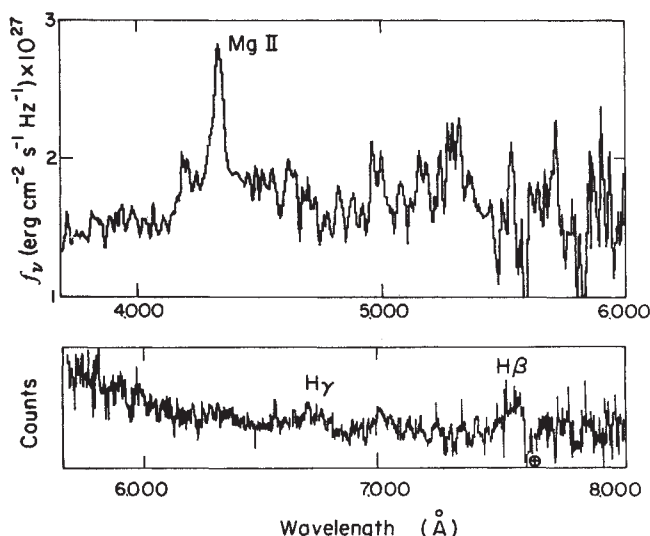


Fig. 1 The spectrum of the QSO 0015+162. The features at 5,577, 5,890 and 7,243 Å are caused by imperfect subtraction of the night sky emission lines.

There is tentative evidence³ for a large supercluster of galaxies at about this redshift, in an adjacent field in SA 68, to the south-west of the QSO and the cluster 0016+16. It would be artificial to speculate that the QSO, which is at the extreme opposite end of the supercluster structure, has a velocity perfectly appropriate for the cluster, but is instead a non-member accidentally seen in projection. We thus believe that the QSO is not only a member of the supercluster, but also of the rich cluster 0016+16. As such it is the best evidence so far for QSO membership in a rich cluster: the cluster is directly observed, not inferred, and its properties were well established before discovery of the QSO.

There are several interesting implications associated with the presence of this ordinary QSO in the cluster. First, we can confirm the redshift of this important cluster, which has previously been based only on the velocity of one member² and inferences from galaxy colours coupled with evolutionary models³. Second, because the redshift of the cluster has been independently inferred strictly through diagnostics based on starlight (galaxy colours and spectral absorption features), the association of this luminous QSO with this very distant cluster is further support for the argument for a cosmological interpretation of QSO redshifts. Further, it seems clear that QSOs do not necessarily avoid the environment of rich clusters. We previously noted¹ that the absence of QSOs in rich clusters could have profound implications for the physical nature of QSOs, or simply be a reflection of the difficulty of observing high redshift clusters. The fact that 0016+16 is one of the most intensively studied high redshift clusters, and is now the first to be noticed as the host of a QSO, implies that the latter of the two alternatives is correct, and that further cases will be forthcoming. If such associations were instead very rare, it would be fortuitous that this particular cluster was selected for intensive study.

If the cluster is dynamically relaxed (which is not obvious due to its elongated structure³), then the location of the QSO with respect to the cluster core provides a crude upper limit on the mass of the QSO: the object obviously does not dominate the mass of the cluster. In any case, the empirical fact that QSOs in rich clusters need not be well centred adds credence to the suggestion¹ that 3C345 and QSO 1641+3998 constitute an additional case of QSOs in high redshift clusters. It has previously been noted that emission line galaxies are seldom found near the centre of rich clusters¹⁶, presumably due to sweeping of gas from the central galaxies. Bothun *et al.*¹⁷ have explicitly predicted that any QSO members of rich clusters will similarly be found outside of the cluster cores, and at least for

these two X-ray selected cases we now verify this prediction. An additional example of an X-ray selected QSO located on the outskirts of a rich cluster may be the object⁷ 0037+061, which has independently been designated¹⁸ member no. 3 in Abell 76; however, further observations are required to establish the redshift of that cluster with confidence.

The QSO 0015+162 provides an interesting opportunity for a unique probe of the physical state of the intracluster gas that is common in rich clusters. The characteristics of the hot gas have been well studied through its diffuse X-ray emission. However, constraints on any cold component of this gas are much weaker. Although the absorption lines commonly found in the spectra of high redshift QSOs are often ascribed to intervening galaxies and/or clusters¹⁹, the absorber is seldom visible. Bright QSOs in or behind visible rich clusters provide a potential probe through the opportunity to search for absorption lines in the QSO continuum caused by the intracluster medium. However, not only are such visible QSO/cluster configurations very rare, but normally the cluster redshift is so small that only non-resonance lines of unfavourable cross-sections and/or ionization states, such as Ca H and K or Na D, are available for study in the optical spectrum. In this cluster the redshift is sufficient to bring the very strong Mg II doublet into the easily accessible blue spectral range. Thus studies of the Mg II region of the spectrum of this QSO at high spectral resolution and signal-to-noise may be very interesting; there is indeed already a hint of complex structure in our low resolution spectrum.

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Do galactic bulge X-ray sources evolve into millisecond pulsars?

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The recently discovered¹ pulsar PSR1937+21 in 4C21.53 is remarkable for the shortness of its period ($P = 1.5$ ms) and for the length² of its characteristic age, $\tau_p = P/2\dot{P} \approx 10^5$ yr. The simple explanation that this is an ordinary supernova-formed pulsar but with an unusually weak magnetic field and hence weak magnetic dipole radiation (ref. 3), that is,

$$B \approx 2 \times 10^{10} (\tau_p / 10^5 \text{ yr})^{-1/2} (P / 1.5 \text{ ms}) \text{ G}$$

fits in with the position of the pulsar close to the galactic plane. We suggest here an alternative origin for the pulsar as being the remnant of one of the 'galactic bulge' X-ray binary stars.

The galactic bulge X-ray sources have luminosities of 10^{37} – 10^{38} erg s $^{-1}$ and are thought to be compact objects (presumably neutron stars) accreting material through a disk from a low-mass quasi-main sequence companion star at rates of the order of 10^{-9} – $10^{-8} M_{\odot}$ yr $^{-1}$ (ref. 4). Indirect evidence that some of them are neutron stars (rather than black holes) is given by the observation of X-ray bursts 5 . A neutron star which has a sufficiently weak magnetic field ($B \leq 10^9$ G, ref. 6) that the magnetosphere is crushed down close to or at the stellar surface and which is accreting from a disk would be spun up to a period comparable with a keplerian orbit at the inner disk radius by the accreted angular momentum. The minimum period (~ 1 ms) reached by such a neutron star before it becomes unstable to gravitational wave emission (ref. 7) is attained within the lifetime ($\sim 10^9$ yr) of the X-ray source. We note that if the magnetic field is strong enough to disrupt the disk at a radius much larger than the neutron star, such short rotation periods cannot be obtained. The lack of observed short pulse periods in the galactic bulge sources indicates either that the fields are weak enough not to disrupt the disk and so produce an observable modulation, or that the periods are short enough to be smeared out by the electron scattering region which appears to surround at least some of the sources 8 .

The present mass transfer rate in these systems can only be sustained for $\sim 10^9$ yr and once the rate has dropped sufficiently, the rapidly spinning neutron star can turn on as a pulsar. The presence of a rapid pulsar has been suggested in Cyg X-3 (refs 9, 10) which might be taken as an intermediate case. Discussions of the ultimate fate of low-mass semidetached binaries 11 indicate that as the mass of the mass-losing companion decreases, the star becomes degenerate and the binary period lengthens. It is not clear whether the companion would by now have been fully accreted or perhaps ablated by the pulsar emission or whether a detached very low mass (Jupiter sized) companion might still be present in orbit with a period of several hours.

The space distribution and kinematics of pulsars formed as remnants of low-mass X-ray binaries (population II pulsars) would be radically different from the normal pulsars which are thought to be formed from extreme population I objects. There is even the possibility that such pulsars could form in globular clusters from the low-mass X-ray binaries seen there. At a given luminosity the population II pulsars should have weaker magnetic fields, larger τ_p and shorter periods than their population I counterparts. There are ~ 30 X-ray luminous ($L_x \geq 10^{37}$ erg s $^{-1}$) low-mass X-ray binaries known 12 , with mean X-ray lifetimes of $\sim 10^9$ yr. Assuming a uniform birthrate, the total number of population II pulsars now visible would be $\sim 300f/(\tau_p/10^{10}$ yr) where f is the fraction of such systems which produce pulsars. The detection of more population II pulsars would then imply $\tau_p \geq 10^8$ yr, and $B \leq 10^9$ G. This is consistent with the requirements for spin up to a period ~ 1 ms discussed above.

The arguments presented here put forward the idea of a new class of radio pulsars which have abnormally weak magnetic fields and which are distributed around the galactic centre. The discovery of stable millisecond pulsations in galactic bulge or globular cluster X-ray sources would lend weight to these ideas as would the detection of Doppler shifts due to very low mass detached companions of such pulsars.

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Note added in proof: The paper by Alpar *et al.* 13 has been brought to our attention since submission of this letter. Our conclusions are consistent with theirs.

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Simultaneous IR and optical light curves of 2A0311–227

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The complex wavelength dependence $^{1-4}$ shown by the light curves of the AM Herculis type binaries such as 2A0311–227 (EF $_4$ ERI) and AM Her itself has led to suggestions $^{5-7}$ that accretion occurs onto both magnetic poles of the white dwarf. The field strengths of the two poles being sufficiently different that one pole dominates at optical wavelengths, while the other produces most of the emission in the IR. Other authors 3 have argued for a single pole model explaining the wavelength dependence of the light curves in terms of the rapidly changing cyclotron opacity with wavelength. An observational method of distinguishing between these two models is provided by the rapid flickering, which is a characteristic property of the light curves at both optical and IR wavelengths. We report here that the optical and IR flickering of 2A0311–227 are highly correlated indicating that in this object the dominant source of cyclotron radiation at both wavelengths is the same accretion column.

2A0311–227 was observed on 9 January 1981 with the 3.8-m UK IR telescope at Mauna Kea. The simultaneous IR–optical photometer described by Bailey *et al.* 8 was used. The IR channel used a J filter (1.25 μ m). The optical channel used an unfiltered GaAs tube. The response is modified by the dichroic beam splitter used to separate optical and IR beams. This reduces the red response of the photometer giving an effective wavelength near 0.44 μ m and a bandwidth of 0.3 μ m. Data were obtained with integration times of 4.8 s covering just over two cycles of the 81-min binary period. The light curves are shown in Fig. 1, where the data have been binned to 24 s per point.

The light curves at J resemble previous data $^{2-4}$ in showing a well defined narrow and broad minimum during each cycle. The narrow minima have been used previously 3,4 to derive an ephemeris for the system. The two additional times of minimum from the present data more than double the baseline, and give the following revised ephemeris:

$$T(\text{IR min}) = \text{JD } 2443944.9519 + 0.056265923E \\ \pm 0.0003 \pm 0.000000019$$

The binary light curve observed in the optical channel is clearly very different from that in the IR as indicated by previous (non-simultaneous) data. In particular there is a well defined narrow minimum in the IR light curve which is not accompanied by a feature of comparable depth in the optical. There are some low points at this phase in the optical curve which at first sight might be attributed to a corresponding shallower and even narrower minimum than that seen in the IR. However, inspection of the individual cycles shows that the feature is neither reproducible or at the same phase from cycle to cycle. Furthermore, for the data shown, the optical feature is not statistically distinguishable from the flickering at the 94% significance level, using a variety of non-parametric tests. We therefore consider that the most likely explanation of these low points is simply

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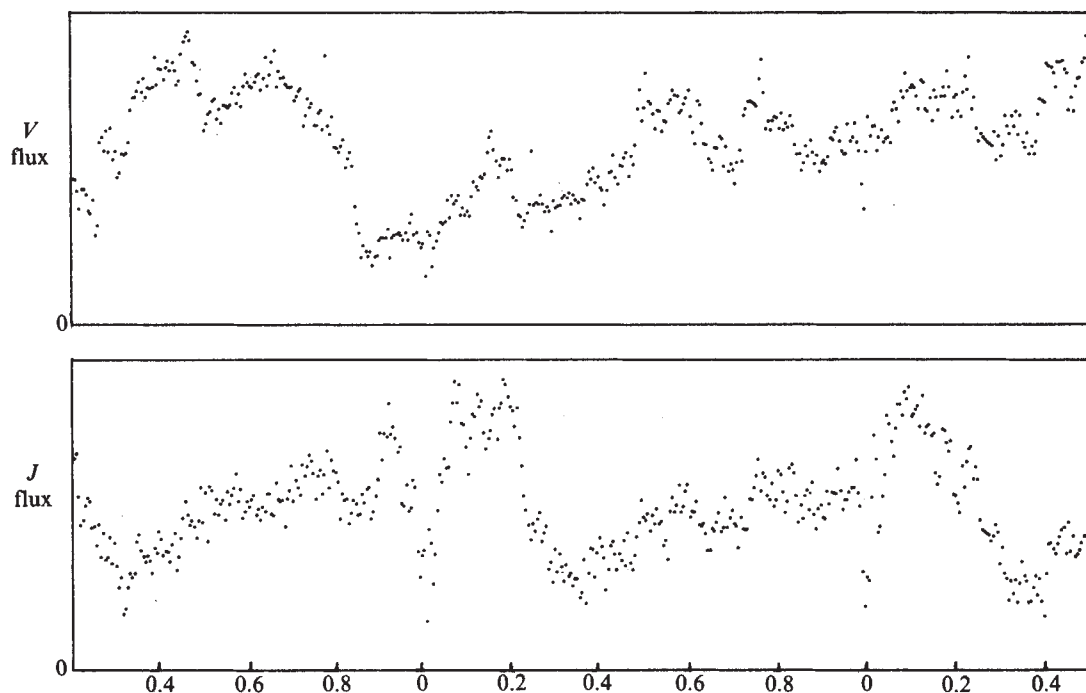


Fig. 1 Light curves of 2A0311-227 obtained on 9 January 1981 in broad band optical ($0.55\ \mu\text{m}$) and IR J band ($1.25\ \mu\text{m}$). The data are plotted on a linear intensity scale.

the flickering activity. The difference in optical and IR light curves at this phase are clearly real and cannot be explained by night-to-night variations as suggested by Motch *et al.*⁹

To separate the flickering component of the variability from that associated with the binary cycle the data were high pass filtered by subtracting from the data set a smoothed light curve obtained by taking the running mean of N points. The autocorrelation (ACF) and cross-correlation (CCF) functions of the filtered data were then calculated. Figure 2 shows the ACFs and CCF calculated from the data of Fig. 1 with $N = 60$. The peaks in the ACFs show that flickering is present in both optical and IR light curves. The similar peak in the CCF indicates that a substantial amount of the flickering is correlated between the IR and optical light curves. Similar calculations were made for a range of values of N , but all show essentially the same cross correlation at zero delay, the different degree of filtering only changing the width of the peak.

Figure 3 shows the CCFs obtained from the original 4.8-s resolution data broken up into four sets of length 40 min. Correlated flickering is clearly present throughout the run, despite the fact that the amount of IR flickering is smaller during the broad minimum.

A 40-min observation of a twelfth magnitude constant star was made soon after the observation of 2A0311-227. An identical analysis of these data revealed no correlation between the optical and IR light curves, with the ACFs and CCF being consistent with random noise. This test was made to check that no spurious correlation could be present in the 2A0311-227 data as a result of guiding errors, seeing effects, or photon noise.

Flickering in both normal cataclysmic variables and in AM Her binaries is normally attributed to variations in the release of accretion energy due to inhomogeneities in the mass transfer stream. In the case of AM Her correlations between flickering in the photometric and polarization data^{10,11} demonstrate that the flickering radiation originates in the strong field region close to the white dwarf's magnetic poles. In a two-pole model for an AM Her type binary the gas streams accreting onto the two poles have travelled very different paths and the radiation from the two accretion columns could not exhibit flickering which is correlated at zero delay.

Our observations therefore indicate that the bulk of the cyclotron radiation at both optical and IR wavelengths origi-

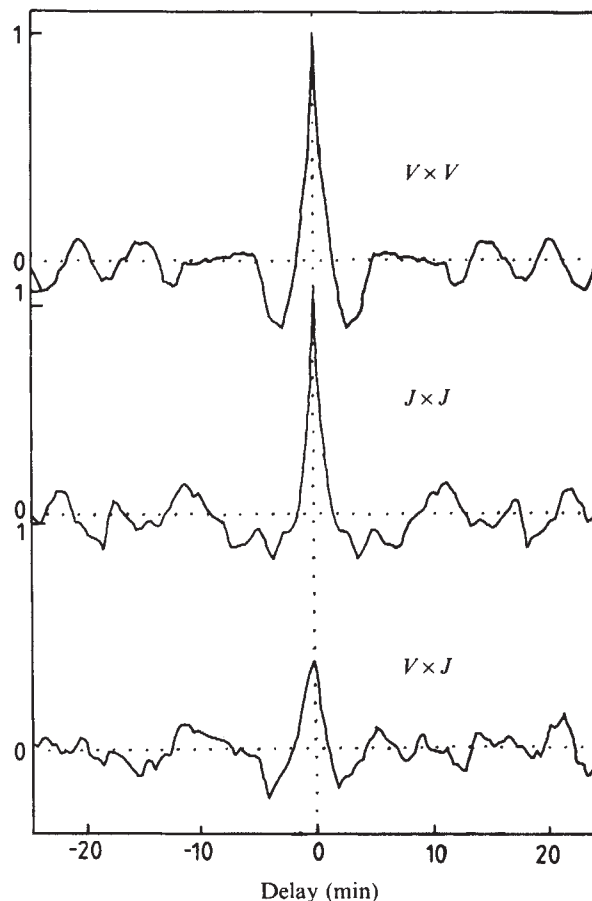


Fig. 2 Autocorrelation function of optical data ($V \times V$), autocorrelation function of J data ($J \times J$) and cross-correlation function of optical and J data ($V \times J$) calculated from the light curves shown in Fig. 1. The delay is in the sense that for a positive delay in ($V \times J$) J follows V .

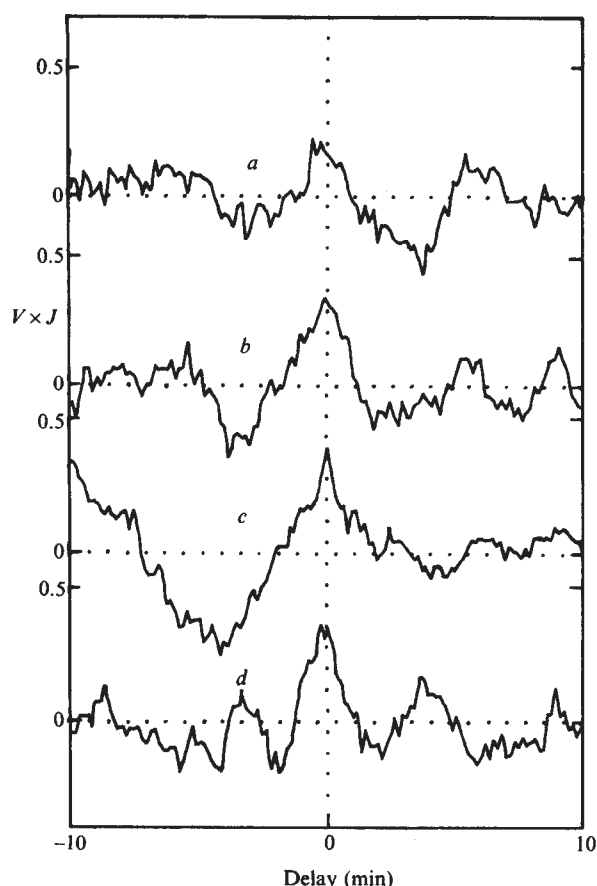


Fig. 3 Cross-correlation functions of optical and IR data broken up into four sets of length 40 min each. The sense of delay is the same as Fig. 2.

nates from the same magnetic pole of the white dwarf. Since the X-ray light curve of 2A0311-227 (refs 12, 13) is very similar to that in the IR³, it is probable that the X rays also originate from the same pole. The amount of accretion energy released at the second pole must therefore be very small.

The large variations in the form of the binary light curve with wavelength must therefore be explained in terms of a single accretion column. As discussed by Bailey *et al.*³ the changing cyclotron opacity with wavelength is probably responsible for this, and also explains other aspects of the observations such as the decreasing polarization with increasing wavelength.

The lack of any significant time lag ($\Delta t < 10$ s) between the optical and IR flickering places a limit on the vertical separation of the emitting regions in the accretion column, if the stream velocity is known. For free fall velocities the maximum separation is a few $\times 10^9$ cm.

Liquid water and active resurfacing on Europa

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Voyager images of the jovian moon Europa show a bright icy surface transected by a complex pattern of long, linear fracture-like markings with a slightly lower albedo¹. Very few impact craters are observed, with sizes generally in the range 10–20 km. Indicators of surface composition and texture include deep IR H₂O absorption features², UV absorption features characteristic of sulphur in a water matrix on the trailing hemisphere³, and a photometric function indicating much more homogeneous scattering than areas of equal albedo on Ganymede and Callisto⁴. Europa's mean density is 3.03 g cm^{-3} , indicating a primarily silicate composition. However, if the density of the silicates is the same as for Io, the H₂O mass fraction would be $\sim 6\%$, enough to form an outer layer of H₂O > 100 km thick if completely differentiated. Several models have been suggested for the evolution and present state of Europa^{5–8}. We present here arguments for recent resurfacing by H₂O from a liquid layer, based on new interpretations of recent spacecraft and Earth-based observations and revised theoretical calculations.

Several heat sources have influenced Europa's evolution. Accretional heating could have been important in dehydrating silicates or melting ice in the outer portion of the body early in its history. Radiogenic heating for assumed chondritic abundances of U, K, and Th would create a current equilibrium surface heat flow of $\sim 8 \text{ erg cm}^{-2} \text{ s}^{-1}$. The present radiogenic heat flow is probably greater due to delayed transport of radiogenic heat produced early at higher rates. If, as our calculations suggest, a silicate core is overlain by an ocean of liquid water and a thin shell of ice I, tidal heating should act in both core and shell. Using parameters appropriate for silicates and ice, and the technique of Cassen *et al.*⁹, we find the mean surface heat flow from tidal dissipation in the core is $\sim 16 \text{ erg cm}^{-2} \text{ s}^{-1}$, and that from the shell is $\sim 28 \text{ erg cm}^{-2} \text{ s}^{-1}$. The mean total surface heat flow density would therefore be $\sim 52 \text{ erg cm}^{-2} \text{ s}^{-1}$, sufficient to maintain a mean shell thickness of ~ 16 km if heat transport was by conduction in solid ice. This total is greater than calculated previously by Cassen *et al.*⁷, because they neglected dissipation in the core and used a dissipation function of $Q = 100$, compared with the value of 25 used here. We have adopted this value of Q for tidal periods because it is appropriate for long period oscillations of the Moon^{10,11}, and is also consistent with the observed high heat flow from Io^{12–14}. Rigidities used are $3 \times 10^{11} \text{ dyn cm}^{-2}$ (ref. 15) for silicates and $4 \times 10^{10} \text{ dyn cm}^{-2}$ (ref. 16) for ice. If Europa's ice shell behaves viscoelastically over tidal periods, not essentially elastically as assumed here, we estimate that the shell dissipation rate would be comparable to but possibly greater than that given above.

Below its dehydration temperature, an H₂O-containing silicate such as serpentine shows strength and effective viscosity comparable with those of dry silicates, and is not expected to convect in the solid state. Viscosities of water-rich silicates may decrease significantly at the dehydration temperature as a consequence of formation of a separate water phase¹⁷. Although such viscosities might be low enough to permit convection, this convection could take place only after separation of the water-phase, and could not maintain temperatures below the dehydration temperature and prevent removal of water from the deep interior. Taking the temperature at the top of the silicates to be $\sim 273 \text{ K}$, the calculated radiogenic and homogeneous tidal heat production implies that water of hydration is retained in

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the outer ~60 km of the silicates. Even with complete serpentinization of this region, an H₂O abundance consistent with Europa's density implies an outer water/ice layer many tens of kilometres thick.

If solid state convection could occur in the ice shell, heat transport rates would be sufficient to freeze the underlying water¹⁸ rapidly. Using an ice activation energy constant of $E/kT_{\text{melting}} = 26$ rather than the value of 18 used previously⁶, solid state convection could only occur for ice crusts thicker than about 30 km, however. The larger value is favoured by Weertman¹⁹, and is consistent with observations of crater relaxation on icy satellites²⁰. Again taking a thermal conductivity appropriate for solid ice ($3.4 \times 10^{-5} \text{ erg cm}^{-1} \text{ K}^{-1}$), we expect a thin (<30 km) shell of ice underlain by liquid water to exist at present if the surface heat flow has never been lower than $\sim 20 \text{ erg cm}^{-2} \text{ s}^{-1}$.

These calculations are sensitive to parameter uncertainties, particularly because the heat flux is comparable in magnitude with that required to maintain a liquid layer. Our parameter estimates are consistent with the presence of such a layer. For confirmation we must look to the observational evidence.

The paucity of craters on Europa implies a mean retention time for 10 km craters of only 3×10^7 yr based on an impact flux calculated from the expected collision rate with comet nuclei²¹. Using appropriate relationships for relaxation of topography with temperature dependent viscosity²² and a geothermal gradient given by our calculated heat flow, and making the very conservative assumption that a crater will become 'invisible' once its floor rebounds by a factor of 1/e, this retention time implies a surface viscosity corresponding to a temperature of ~140 K. This value is much higher than the mean solar equilibrium temperature of ~92 K, and actually must be higher still to relax crater rims. To allow high near-surface temperatures, even for very high heat flows, an insulating blanket at the surface is required. Fracturing of a thin ice crust over liquid water could provide such a blanket. Water exposed by fracturing would not flood the surface, due to the buoyancy of the crust, but would boil, producing vapour that would condense as frost over a large area. Frosts typically have very low densities and thermal conductivities, and could provide the insulation required. An insulating layer could also result in an average crustal thickness much less than the mean value of ~16 km calculated for conduction in solid ice alone.

There is evidence for such a frost layer. First, the photometric function of Europa requires much more homogeneous scattering than produced by ejecta deposits of equal albedo on Gany-mede and Callisto⁴. The difference is probably textural, and is consistent with a more tenuous structure than produced by impacts. Second, deposits of sulphur, evidenced by a UV absorption feature³, are only present on the trailing hemisphere. This feature is attributed to S-O bonds, and a sulphur column density of $2 \times 10^{16} \text{ cm}^{-2}$ is inferred. Estimated values for the flux of S ions into Europa's trailing hemisphere predict that this column density would be deposited in only ~7 yr. These observations were interpreted to require equilibrium between deposition of S ions and escape of SO₂ molecules by charged particle sputtering, since H₂O escapes more readily than SO₂ (ref. 23). Recent measurements²⁴ have shown that SO₂, which is more volatile than H₂O at thermal energies, is more easily sputtered than H₂O by high-energy particles. As SO₂ also has a lower escape rate, such a model would imply higher abundances on the leading hemisphere where sputtering is much less severe, contrary to observations. Assuming instead that the observed column density is a result of uniform addition of S ions to a surface on which a much larger amount of H₂O is continually deposited, an estimate of the minimum H₂O deposition rate can be obtained. For a UV measurement depth of 5,000 Å, this rate would be $\sim 0.1 \mu\text{m yr}^{-1}$.

The appearance of Europa is consistent with this model. Vapour released from fractures will travel large distances, while particulate matter such as salts or organic material would be preferentially deposited near an open active surface. Such

material could also undergo radiation darkening²⁵, creating a pattern of darker features on a bright surface. Stresses sufficient to fracture the crust could be generated by tidal flexure^{6,26}, or by membrane stresses induced by rotation of the ice shell over Europa's tidal bulge. The crust would in reality be inhomogeneous in thickness, and fractures would form preferentially in thinner regions. Continuing or repeated deformation would cause repetitive activation of pre-existing fractures. If large resurfacing events are frequent on Europa they might be observable as transient increases in the thermal IR flux or as clouds of vapour and condensate visible to the Galileo orbiter.

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Metamorphic fluids in the deep crust: evidence from the Adirondacks

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Unusual mineral assemblages of tremolitic hornblende + diopside + enstatite + quartz have been examined to estimate the temperature (T) and water fugacity ($f_{\text{H}_2\text{O}}$) at the time of the granulite facies metamorphism in the Adirondacks. The pyroxenes are closely represented by the system CaO-MgO-SiO₂ allowing application of experimental data¹ with only small corrections for reduced values of activities. Tremolite equilibria² buffered $\log f_{\text{H}_2\text{O}} = 3.0 \pm 0.1$, equivalent to 0.9 kbar of water pressure ($P_{\text{H}_2\text{O}}$) at 7 kbar, 710 °C. Nearby marbles contain wollastonite + calcite + quartz and buffered $P_{\text{CO}_2} = 1.1$ kbar, consistent with $P_{\text{H}_2\text{O}} \leq 5.9$ kbar. Inferred local gradients in fluid compositions suggest that deep crustal fluid conditions are complex and heterogeneous indicating that fluid movements, whether of mantle³ or deep crustal origin, are channelized rather than pervasive. Thus some granulite facies rocks were wholly or partly closed systems with respect to externally derived fluids permitting localized buffering of fluid compositions. Recognition of restricted fluid-mixing limits theories that call for massive amounts of pervasive fluid flow to facilitate melting, cause metasomatism, or to stabilize granulite facies mineralogy.

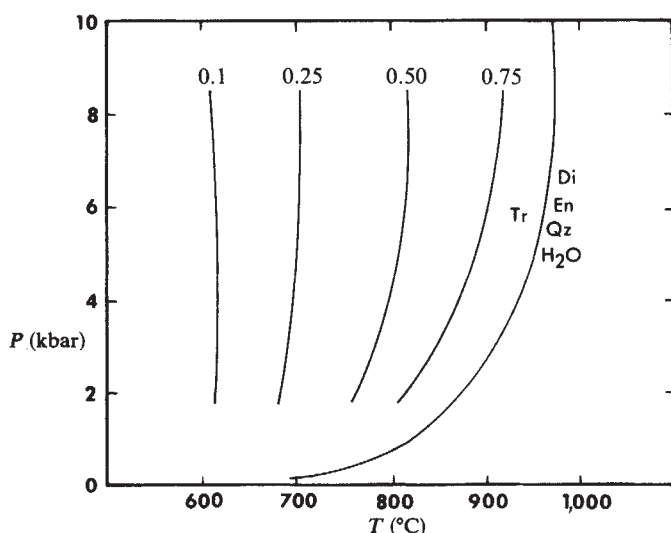


Fig. 1 Dehydration equilibria for synthetic tremolite² for $X_{H_2O} = 0.1, 0.25, 0.50, 0.75$ and 1.0 .

We have found a series of metasediments containing enstatite and diopside located at Wolf Hill (JM/TRD) and Burnham Mountain (ST14) in the south-east Indian Lake Quadrangle in the Adirondack Mountains, New York, USA. These localities are 2–4 km from the contact of the Oregon Dome, a major anorthosite outlier of the Mt Marcy anorthosite massif⁴. Detailed mapping and structural analysis show the upper contact of the Oregon Dome to dip shallowly beneath these metasediments⁴, suggesting the possibility of early contact metamorphism. While this may have occurred, the equilibria now preserved are consistent with P - T conditions at the peak of regional metamorphism during the 1,000-Myr Grenville orogeny. Any contact aureole that formed during intrusion of the anorthosite has thus been overprinted by the subsequent granulite facies metamorphism. This is supported by textural relationships, showing undeformed, poikiloblastic grains of enstatite and diopside growing across well-defined planes of foliation. This foliation is regional and occurs throughout the nearby anorthosite⁴; which, therefore, predates the assemblages under consideration. This conclusion is consistent with radiometric dates that suggest anorthosite intrusion some 100–200 Myr before regional metamorphism^{5,6}.

The P - T conditions of granulite facies metamorphism in the Adirondacks are well known from several studies. Isotherms have been contoured using temperature estimates from re-integrated feldspar and oxide compositions⁷. These temperatures are in good agreement with those estimated from silicate reactions^{8,9}. Pressures have been estimated from the common occurrences of sillimanite¹⁰ and rare kyanite^{11,12}, from ferrosilite and fayalite-quartz assemblages¹³, from a rock containing akermanite-monticellite-wollastonite⁹, and from various garnet assemblages^{14,15}. Taken together, these data from differing rock types and widely spaced localities are self-consistent and indicate that $T = 710 \pm 30^\circ\text{C}$ and $P = 7 \pm 1$ kbar for the peak of regional metamorphism in south-east Indian Lake Quadrangle.

Minerals were analysed by electron microprobe using standard procedures for analysis, data reduction and cation normalization⁸. Representative analyses are given in Table 2. The pyroxenes are remarkably close to the diopside-enstatite join with 1–2% Al_2O_3 as their greatest impurity. Enstatites contain 92–97 mol % MgSiO_3 and diopsides contain 88–94 mol % $\text{CaMgSi}_2\text{O}_6$. Phlogopites are slightly peraluminous with 13.1 wt % Al_2O_3 . Approximate whole-rock chemical analyses (Table 2) were calculated from modes (Table 1), the mineral analyses and estimated densities.

The solvus between enstatite and diopside has been experimentally reversed at various P - T (ref. 1), but none of the experimental data is greatly restrictive below 15 kbar and

Table 1 Mineralogy and modal analysis (1,000 points)

	JM/TRD-2	ST-14-1
Quartz	56.8	3.4
Diopside	11.2	54.5
Enstatite	1.0	21.8
Amphibole	31.0	—
Phlogopite	—	14.1
Pyrrhotite	—	2.6
Apatite	—	0.1
Plagioclase	—	3.5

850 °C. Margules formulations have been fit to the pyroxene data with a maximum discrepancy of 18 °C between model and experiment¹⁶. Application of these equations to the pyroxene pairs, using the Wood-Banno model¹⁷ for distributing minor cations, yields $T = 278$ – 625°C (Table 3). In this temperature range the solvus is steep and very sensitive to small analytical, experimental or thermodynamic errors. Routine errors in microprobe analysis alone could account for the discrepancy between these values and the inferred metamorphic T suggesting that the pyroxene solvus, as applied here, is not precise enough to be useful for metamorphic thermometry. Alternatively, it could be proposed that these temperatures accurately represent retrograde reequilibration of pyroxenes, but our data do not resolve this.

The calculated rock analyses (Table 2) are unusual with high Mg, $\text{Mg} > \text{Ca}$, low Fe and variable Si. Talc-tremolite-quartz schists near Balmat in the north-west Adirondacks have similar chemistry and are associated with halite and anhydrite-bearing marbles that are clearly metaevaporites¹⁸. The samples of this study may thus represent isochemical metamorphism of evaporites or they may have been metasomatized, possibly by nearby anorthosite.

Many reactions can be written for the dehydration of a complex natural amphibole to a pyroxene-bearing assemblage. The simplest end-member reaction that has been experimentally calibrated² is:



Our sample, JM/TRD, contains this H_2O -buffering assemblage. Figure 1 shows this reaction calculated for various values of X_{H_2O} . Reaction positions were calculated from the equilibrium point at $P_{H_2O} = 2$ kbar, $T = 875^\circ\text{C}$ (ref. 2) using the computer program EQUIL⁸. To apply reaction (1) to the assemblage of JM/TRD a correction must be made for the effects of solid solution in both the amphibole and pyroxenes. In the absence of calibrated activity versus composition relations this correction can be approximated using an ideal ionic model^{8,17}. This results in a change of Gibbs energy of reaction (1) of -4.8 to -6.8 kJ due primarily to fluortremolite and tschermakite solid solution in amphibole. The range of energies derives from different amphibole models with variable order-disorder on various cation sites and extends the stability of amphibole 75–100 °C, consistent with experiments on tschermakite¹⁹. At 7 kbar, 710 °C these compositions thus buffer $X_{H_2O} = 0.11$ – 0.14 or $\log f_{H_2O} = 3.0 \pm 0.1$ (Fig. 1). A larger uncertainty in the application of these experiments arises from results showing that some synthetic tremolites decompose more than 100 °C below natural tremolite, possibly due to crystal defects accounting for up to 8 kJ change in Gibbs energy²⁰. In extreme cases, a correction for the effect of crystal defects in the experimental charge would shift estimated X_{H_2O} from 0.11 to 0.05 while high contact metamorphic temperatures of 850 °C would shift estimated X_{H_2O} from 0.11 to 0.29. Low P_{H_2O} is indicated in any case.

Considerable controversy exists as to the significance of low P_{H_2O} values in high grade metamorphic rocks. It has been proposed that low P_{H_2O} results from dilution of a fluid phase by deep-seated CO_2 (refs 3, 21) or CH_4 (ref. 22) of either crustal or mantle origin or that alternatively there was no free fluid phase²³. A traditional alternative view regards

Table 2 Electron microprobe analyses of minerals and calculated rock analyses

	JM/TRD-2				ST-14-1			
	Opx	Cpx	Amph	Rock	Opx	Cpx	Phlg	Rock
SiO ₂	58.81	54.55	53.00	80.99	59.97	55.79	42.07	52.19
TiO ₂	<0.05	0.17	0.71	0.27	<0.05	0.12	2.79	0.40
Al ₂ O ₃	1.54	1.99	7.29	2.50	0.87	2.01	13.10	3.58
Fe ₂ O ₃ *	0.00	0.76	0.00	—	0.08	0.00	0.00	—
FeO*	2.63	0.00	0.78	0.39	0.74	0.24	0.35	3.25
MnO	0.18	0.07	<0.05	0.01	0.20	0.12	<0.05	0.11
MgO	36.73	18.84	21.01	10.09	39.54	17.40	27.13	21.05
CaO	0.23	24.91	12.91	4.41	0.17	24.64	<0.05	13.54
Na ₂ O	<0.05	0.21	0.82	0.31	<0.05	0.45	0.23	0.45
K ₂ O	ND	ND	0.75	0.26	ND	ND	10.22	1.25
H ₂ O*	ND	ND	1.85	0.63	ND	ND	3.46	0.41
F	ND	ND	0.69	0.24	ND	ND	1.72	0.20
Cl	ND	ND	<0.05	<0.01	ND	ND	<0.05	<0.01
Total†	100.12	101.50	99.52	100.00	101.57	100.77	100.35	100.00‡
Si	1.996	1.934	7.325	—	1.982	1.997	2.935	—
Al	0.004	0.066	0.675	—	0.018	0.003	1.065	—
Al	0.056	0.017	0.510	—	0.016	0.081	0.012	—
Ti	<0.002	0.005	0.073	—	<0.002	0.003	0.146	—
Fe ³⁺ *	0.000	0.020	0.000	—	0.002	0.000	0.000	—
Fe ²⁺ *	0.074	0.000	0.090	—	0.020	0.007	0.020	—
Mn	0.006	0.002	<0.006	—	0.006	0.003	<0.006	—
Mg	1.858	0.998	4.327	—	1.950	0.928	2.821	—
Ca	0.008	0.946	1.912	—	0.006	0.945	<0.003	—
Na	<0.003	0.014	0.220	—	<0.003	0.031	0.031	—
K	ND	ND	0.132	—	ND	ND	0.909	—
Cl	ND	ND	<0.006	—	ND	ND	<0.003	—
F	ND	ND	0.300	—	ND	ND	0.381	—
OH†	—	—	1.700	—	—	—	1.619	—

ND, not determined. * Calculated from normalized formula. † Adjusted for Fe₂O₃, F = 0 and H₂O. ‡ Includes 3.61 SO₃, 0.04 P₂O₅.

Table 3 Calculated activities of MgMgSi₂O₆ and CaMgSi₂O₆ in pyroxenes and calculated equilibrium T†

	JM/TRD	ST-14
* $\alpha_{\text{MgMgSi}_2\text{O}_6}^{\text{opx}}$	0.863	0.951
* $\alpha_{\text{CaMgSi}_2\text{O}_6}^{\text{opx}}$	0.007	0.006
* $\alpha_{\text{MgMgSi}_2\text{O}_6}^{\text{cpx}}$	0.037	0.018
* $\alpha_{\text{CaMgSi}_2\text{O}_6}^{\text{cpx}}$	0.907	0.860
† T _A (°C)	451	278
† T _B (°C)	557	625

* From ref. 17.

† Using equations A and B of ref. 16.

low $P_{\text{H}_2\text{O}}$ values as anomalous and assumes that in general $P_{\text{H}_2\text{O}} = P_{\text{lithostatic}}$.

The samples of this study occur in proximity to granulite facies, wollastonite-bearing marbles that buffered metamorphic fluids to low P_{CO_2} . Sample SP202-1 was also collected from Burnham Mountain, 1 km on strike from ST-14 and contains the assemblage wollastonite + calcite + quartz + grossular + sphene + apatite⁸. Grossular (Gr₈₈) + quartz in this sample limits temperature to below 815 °C at 7 kbar (ref. 9), further arguing against high temperature contact metamorphism. The assemblage wollastonite + calcite + quartz buffers P_{CO_2} to 1.1 kbar. Similar low values of P_{CO_2} are inferred for 30 other wollastonite localities that are known in the Adirondacks including the mines near Willsboro, New York that account for most of the world wollastonite production^{8,9,12,24}.

If the fluids of granulite facies metamorphism are pervasive and migrate in sufficient quantity to control buffering reactions^{3,21}, then the fluid compositions inferred in marbles should be similar to those in the nearby two-pyroxene rocks. This

would indicate that both $P_{\text{H}_2\text{O}}$ and P_{CO_2} were low during Adirondack metamorphism requiring large quantities of some other fluid diluent in the lower crust. Alternatively, we believe that $P_{\text{H}_2\text{O}}$ and P_{CO_2} can be highly variable in marbles and sharp gradients in fluid composition can exist⁸. Such a model suggests great complexity in fluid compositions of the deep crust that is not generally recognized. Locally high $P_{\text{H}_2\text{O}}$ and low P_{CO_2} in marbles can stabilize wollastonite while nearby rocks might have low $P_{\text{H}_2\text{O}}$ stabilizing the two-pyroxene assemblages reported here. Recognition of complex fluid conditions would indicate that generalities should be made with caution as to the composition of fluids in the granulite facies. Such fluid movement as exists in the deep crust may largely be channelized and restricted to zones of weakness, structural misfit or permeable lithologies.

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Mantle reservoirs and ocean island basalts

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The mantle part of the continental lithosphere possesses in part Sr and Nd isotopic compositions similar to those of ocean island basalts. Here we investigate circumstances under which this mantle material may become detached from continental lithosphere, incorporated into the convective flow and thereby providing a source for some ocean island basalts.

Early work on the isotopic compositions of lead and strontium in oceanic basalts demonstrated that the spreading ridges produce material with comparatively uniform $^{87}\text{Sr}/^{86}\text{Sr}$ and $^{208,207,206}\text{Pb}/^{204}\text{Pb}$ ratios, whereas the ocean island basalts exhibit more variability¹⁻⁶. Most samples of mid-ocean ridge basalt (MORB) have low $^{87}\text{Sr}/^{86}\text{Sr}$ ratios (<0.7030) but nevertheless larger than could have been generated with the present Rb/Sr ratios during the history of the Earth. Hence the source of these basalts appears to have been depleted, probably by a melting event at some earlier time. The timing of this event is not clear, since neither the $^{87}\text{Sr}/^{86}\text{Sr}$ at the time of the event, nor the subsequent Rb/Sr ratio, are known.

The availability of accurate measurements of $^{143}\text{Nd}/^{144}\text{Nd}$ and Sm/Nd ratios have strongly influenced these arguments because the relative abundances of Sm and Nd within the Earth differ little from those in chondritic meteorites⁷⁻⁹. The current estimate for the present-day ratio ($^{143}\text{Nd}/^{144}\text{Nd}$)_E for the whole Earth is based on measurements made on meteorites¹⁰⁻¹² and used as a reference to define ϵ_{Nd} for any sample (see Fig. 1 legend). Because partial melting of peridotite, which has $\epsilon_{\text{Nd}} = 0$ for example, produces greater depletion of the residuum in Nd than Sm, the subsequent decay of ^{147}Sm will lead to $\epsilon_{\text{Nd}} > 0$. Nd isotope measurements from oceanic basalts^{10,13-16} showed that $\epsilon_{\text{Nd}} > 0$ for a large number of MORB and ocean island samples. The only exceptions were the Tristan da Cunha basalts with average ϵ_{Nd} close to -2 . This observation led to the suggestion that there were two or more reservoirs within the Earth with distinct isotopic signatures represented by MORB with $\epsilon_{\text{Nd}} \approx 9$ and undepleted Earth with $\epsilon_{\text{Nd}} \approx 0$. The linear correlation between ϵ_{Nd} and $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of MORB and ocean island basalts^{13,14,17} was further used to estimate the bulk Earth $^{87}\text{Sr}/^{86}\text{Sr}$ ratio from the intercept of this line with $\epsilon_{\text{Nd}} = 0$. Our purpose here is to suggest that the view of depleted and undepleted reservoirs in the Earth expressed in this latter estimate is an oversimplification, as has been clearly the case as far as the behaviour of U and Pb in the mantle is concerned¹⁸⁻²². An alternative model is suggested which is consistent with both presently available data and our understanding of the rheological properties of the mantle.

The possibility that at least one reservoir with ϵ_{Nd} substantially less than zero is involved in the generation of oceanic basalts became clear when Dosso and Murthy²³ obtained $1.6 > \epsilon_{\text{Nd}} > -5.5$ for volcanics from Kerguelen. Support for the existence of a mantle reservoir with such characteristics is provided by some of the measurements^{24,25} made on separated clinopyroxene from ultramafic xenoliths in kimberlites and basalts with $0 \geq \epsilon_{\text{Nd}} > -26$. The same correlation between ϵ_{Nd} and $^{87}\text{Sr}/^{86}\text{Sr}$ ratio exhibited by oceanic basalts applies with one exception²⁵ to the results from the xenoliths, although most of them are samples of sub-continental mantle and some may be sub-continental lithosphere. A compilation of results from oceanic islands and MORB are compared with those from ultramafic xenoliths in Fig. 1. It is evident that the values for xenoliths extend from positive values only slightly less than those of MORB to negative values more extreme than those from any oceanic rocks measured so far.

The question of the location of a less-depleted reservoir than that supplying MORB has been a common interest of geophysicists and geochemists for some time. Some geophysicists²⁶⁻²⁹ believe that heat is carried by convection from the core-mantle boundary to the base of the plates and that there is no boundary within the mantle across which heat is conducted. If isotopically anomalous regions are to exist within such a convective system they must do so as blobs of undepleted material³⁰. However, numerical simulations of convective flows suggest that such blobs would be transformed into schlieren or streaks within a few hundred million years^{31,32}. Although these schlieren retain their distinct isotopic characteristics, they are not a plausible source region for the large volumes of basalt required to construct features such as the Hawaiian Ridge. If the core is discounted as a serious candidate for the relatively enriched reservoir, then the only obvious remaining possibility is the continental lithosphere.

It has been argued on chemical grounds³³⁻³⁵ that whole mantle convection is not readily compatible with the observed depletion in the upper mantle inferred from MORB, which requires a large and well-mixed reservoir to exist beneath all plates. Depletion to the extent observed is difficult to achieve unless a part of the mantle has not been involved in the generation of the continents: estimates for the depleted portion vary between about half to one-third of the mantle³³⁻³⁵. It is difficult to understand how this depleted material can be so widely distributed and so uniform in composition unless it is generated from a vigorously convecting upper portion of the mantle.

This reservoir must have exchanged only a small amount of material with the lower mantle, because most of the continental material was removed from the upper mantle. In such a model the lower mantle provides a source of undepleted material if a small mass flux occurs across the upper-lower mantle boundary. This model has been used either explicitly or implicitly by many geochemists to accommodate the variations in isotopic compositions of Sr, Nd, Pb and He between oceanic basalts, and is compatible with our present understanding of convection both in doubly diffusive systems and in the mantle³⁵. However, such a model obviously cannot explain the existence of mantle material with $\epsilon_{\text{Nd}} < 0$ unless the Sm/Nd ratio of the lower mantle is considerably lower than the chondrite-derived reference value. The (limited) evidence available does not favour such a model^{9,10,36}.

Because with few exceptions both positive and negative ϵ_{Nd} observations for oceanic basalts and ultramafic xenoliths correlate with $^{87}\text{Sr}/^{86}\text{Sr}$ ratios, it is logical to seek one explanation for the majority of observations, rather than two processes, one which leads to $\epsilon_{\text{Nd}} > 0$, and the other $\epsilon_{\text{Nd}} < 0$. This explanation requires a reservoir with $\epsilon_{\text{Nd}} < 0$ as one end-member and the only remaining possibility for this is the continental lithosphere.

There are two parts of the continental lithosphere which could be entrained in the convective circulation: the crust and the mantle part of the lithosphere. The process which has received most attention is subduction of continental crust³⁷⁻³⁹. The geochemical evidence for such subduction occurring on a large scale is not strong^{40,41}, although some elements such as uranium and potassium enter basaltic ocean crust through hydrothermal circulation and low-temperature alteration and are subducted at island arcs. The isotopic signatures of altered oceanic crust and pelagic sediment are evident in some island arc basalts⁴¹⁻⁴³, however, only very small amounts of pelagic sediment are required to impart the observed signatures. The dynamics of direct subduction of the continental crust are also unpromising. The crust is both lighter and weaker than the mantle and therefore will only be carried down with difficulty and in small pieces³⁷. No such problems impede the sinking of the mantle part of the continental lithosphere, which is cooler and therefore denser than the asthenosphere. This process was suggested to explain the tectonics of north-west Greece⁴⁴. Two mechanisms have been proposed by which this process might occur. Bird^{45,46} argued that the whole of the mantle part of the continental lithosphere can detach from the crust and sink in

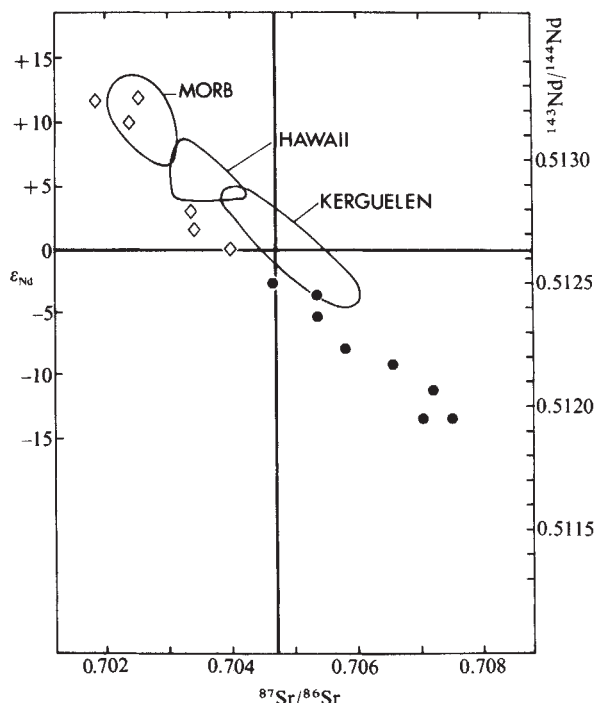


Fig. 1 Comparison of ranges of ϵ_{Nd} ($^{143}\text{Nd}/^{144}\text{Nd}$) and $^{87}\text{Sr}/^{86}\text{Sr}$ ratios for MORB^{13,14,17}, Hawaii¹⁴ and Kerguelen²³ basalts with those recorded for separated clinopyroxene from lherzolite xenoliths from the Kimberley and Bulfontein kimberlites²⁴ (●) and Dreiser Weiher volcanics⁵⁴ (◇).

$$\epsilon_{\text{Nd}} = \left\{ \frac{^{143}\text{Nd}/^{144}\text{Nd}}{(^{143}\text{Nd}/^{144}\text{Nd})_{\text{E}}} - 1 \right\} \times 10^4.$$

where $^{143}\text{Nd}/^{144}\text{Nd}$ is the measured ratio in a particular sample and $(^{143}\text{Nd}/^{144}\text{Nd})_{\text{E}}$ is the estimated value for the whole Earth. The data for MORB, Hawaii and Kerguelen basalts, together with those for other ocean islands such as Iceland, Ascension, Tristan da Cunha, Bouvet¹⁴, were used to define the so-called mantle array^{14,17}. A recent compilation of Sr- and Nd-isotope data for ocean island volcanics may be found in White and Hoffman²². Note that ϵ_{Nd} for ultramafic xenoliths extend to more negative values than oceanic volcanics. A sample of garnet lherzolite from Lashaine²⁵ has an extreme value for $\epsilon_{\text{Nd}} = -26$, and $^{87}\text{Sr}/^{86}\text{Sr} = 0.83$.

much the same way as the oceanic lithosphere sinks beneath island arcs. Such a process remains to be clearly demonstrated for any region; and furthermore it is uncertain whether such behaviour is compatible with the rheological properties of the asthenosphere, which must replace the mantle part of the lithosphere. Houseman *et al.*⁴⁷ have also argued that the mantle part of the continental lithosphere could be detached, but only if the lithosphere had been thickened by shortening in a compressional zone. In areas like the Himalayas thrusting has increased the crustal thickness by a factor of approximately 2. A similar degree of thickening must have occurred throughout the whole lithosphere. Thickening by a factor of 2 increases the thermal time constant by a factor of 4, to about 250 Myr. If the temperature anomaly resulting from such shortening is to be removed by conduction alone, a time comparable with the thermal time constant is required. Houseman *et al.*⁴⁷ have noted that the rapid establishment of regional metamorphic gradients after crustal thickening requires that the temperature anomaly must be removed in much less than 250 Myr. Furthermore, it was shown by those authors that the convective flow necessary to effect the removal of the thermal anomaly can be driven by the negative buoyancy of the thickened lithosphere. The mechanism proposed by Houseman *et al.*⁴⁷, in contrast to Bird's⁴⁵, occurs only in areas where shortening has taken place, and need only involve the portion of the lithosphere which protrudes into the asthenosphere. A sketch of this process is shown in Fig. 2.

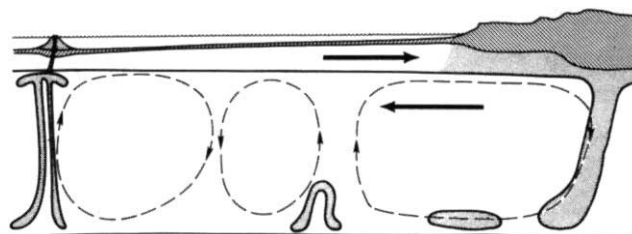


Fig. 2 How the mantle part of the continental lithosphere can produce oceanic island basalts. On the right-hand side the continental crust and lithosphere have been thickened by shortening in an active orogenic zone. The mantle part of the lithosphere, shown dotted, is denser than the mantle below, and is convectively unstable because its thickness is greater than that of the lithosphere elsewhere. It detaches, falls to the base of the upper mantle and is then heated by heat conducted from the lower mantle. The lower boundary layer then becomes unstable and produces hot rising jets. Such jets are known to underlie many oceanic islands and can carry the anomalous material, shown as dotted regions, from the lower boundary to beneath the islands. This circulation is known as the small scale flow. Meanwhile the large scale flow, of which the plate motions form part, has carried the continent elsewhere.

The mantle part of the continental lithosphere is not only easily removed and carried to the base of the convecting layer, but ultramafic xenoliths derived from it demonstrate that at least in part it possesses a Nd and Sr isotope signature that could account for that observed in ocean island basalts (see Fig. 1). The mechanism whereby this part of the lithosphere is produced remains unclear, but in one instance at least it appears to have formed contemporaneously with overlying continental basement ~2,000 Myr ago²⁵. It is not yet possible to estimate whether its rate of incorporation into the convective system is sufficiently rapid to account for a majority of ocean island basalts, but a simple mass balance argument demonstrates that it may be sufficiently 'fertile' to contribute the required quantities of material showing isotopic characteristics typical of ocean islands. For example, the addition of 10% of relatively enriched subcontinental mantle with $\epsilon_{\text{Nd}} = -10$ to a typical MORB source is sufficient to produce a mixture with $\epsilon_{\text{Nd}} = +6$ if the concentration of Nd in the enriched source is twice that of the MORB source. Around 10% of the convecting upper mantle could be modified in this way by the involvement of only 10–15% of the mantle part of the continental lithosphere. A contribution of this magnitude to the convecting upper mantle could be made every few hundred million years if the mantle part of the continental lithosphere survives for 2,000 Myr or more.

Other isotopic systems beside Nd and Sr that have been studied extensively include the isotopes of Pb and He. Pb-isotopic variations reflect fractionation of U and Th relative to Pb, which like Rb are strongly concentrated in the continental crust. Because the concentration of lead is also large in oceanic sediments it is not difficult to produce lead isotope heterogeneities in the mantle by subduction of oceanic crust. For example, the lead isotope signature of subducted oceanic crust can be readily detected in island arc basalts above the sites of crustal subduction^{41–43}. The extent to which Pb-isotopic variations in ocean island basalts reflect such recycling is uncertain, however^{18,22,43}. Whereas most mantle-derived volcanics have $^3\text{He}/^4\text{He}$ ratios higher than the atmospheric value, some ocean islands possess $^3\text{He}/^4\text{He}$ ratios that are 20 to 30 times this value^{48,49}. The only plausible explanation for the excess ^3He in the mantle is that it is primordial, and has remained in the mantle after the atmosphere was produced by degassing. It seems unlikely that the $^3\text{He}/^4\text{He}$ ratio in the silicate mantle part of the continental lithosphere exceeds that of MORB and values between 10^{-5} and 10^{-8} seem likely⁵⁰, although extreme enrichments of ^3He have been recorded in diamonds⁵¹. The high $^3\text{He}/^4\text{He}$ ratios observed in some, but not all, ocean island basalts indicate that contributions have been made to the source

from a portion of the mantle (probably lower) where primordial He has been stored. However, in these situations it is not clear that all of the Sr and Nd are also derived from the same reservoir. One possibility is that He (and possibly other rare gases) are preferentially introduced into the source of some ocean island basalts by diffusion across the boundary between the upper and lower mantle. In this latter case variations in $^3\text{He}/^4\text{He}$ may not necessarily correlate with ε_{Nd} or $^{87}\text{Sr}/^{86}\text{Sr}$ and it will not be possible to explain the observations in terms of simple mixing between reservoirs.

It is noteworthy that seismological observations have demonstrated that considerable variations in velocity occur on length scales of a few kilometres at depths of around 650 km^{52,53}. If this boundary is the division between the upper and lower mantle it is probably sufficiently sharp to allow the diffusion between the two.

Thus, the mantle part of the continental lithosphere has in part Sr, Nd and Pb isotope compositions that render it a potential source of material for some ocean island basalts. The detachment of this mantle portion is likely to occur in regions of tectonic shortening and become incorporated into the convective flow thereafter. Those ocean island basalts with high $^3\text{He}/^4\text{He}$ ratios are only compatible with the mechanism proposed if He and other rare gases are introduced preferentially to Sr and Nd into the source of the ocean island.

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Age of the Banda Sea, eastern Indonesia

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The age of the Banda Sea is critical for palaeogeographical reconstructions of eastern Indonesia. Assumed ages for its formation range from Mesozoic to Neogene^{1–10}. However, geophysical evidence shows that the Banda Sea is floored by oceanic crust^{11,12} with a minimum age of Eocene⁴. Evidence for a sequence of events involving continental breakup and the initiation of spreading that led to the formation of the Banda Sea should be found in the microcontinents around the margins of the sea. Reinterpretation of the geological history of these microcontinents based on new data reported here from western Irian Jaya^{9,13} shows that they separated from Gondwana during the Jurassic and strongly supports the suggestion of Bowin *et al.*⁴ that the Banda Sea is trapped Mesozoic oceanic crust of Indian Ocean affinities.

The Banda Sea is a small marginal sea bordered by microcontinents to the north, Mesozoic ophiolites of east Sulawesi and the Buton microcontinent to the west and the Banda Arc to the south and east (Fig. 1). The structure of its floor has been investigated by several marine geophysical expeditions which concluded that the sea is floored by oceanic crust with up to 1.2 km of sediment^{4,11,12}. Average water depths are between 4 and 6 km although an irregular ridge trending about N 75°E and rising to depths of <2 km divides the sea into the north and south Banda Basins⁴. The sea floor is also characterized by low heat flow values (<1.8 HFU)⁴.

Various palaeogeographical reconstructions of eastern Indonesia have led to suggestions that the Banda Sea was formed by back-arc spreading either during the Neogene^{5,7,10} or (taking into account the sediment thickness³, water depths and heat flow) during late Cretaceous to early Tertiary times. However, the ages suggested by water depths and heat flow must be considered as minimum ages, since the values fall on the asymptotic portion of the Parson and Sclatter¹⁴ curves for age/water depth and age/heat flow for the major ocean basins.

Others have suggested that the Banda Sea is trapped older oceanic crust of Tethyan¹ or Pacific^{6,8} origin cut off by transform faulting related to the westward translation of the Sula Spur, while Bowin *et al.*⁴, based on the trend of limited areas of marine magnetic anomalies, have suggested that it is trapped Indian Ocean floor, that was subsequently displaced westwards during the Tertiary.

New data from regional geological mapping of Western Irian Jaya and the Misool Archipelago by the Indonesian–Australian Irian Jaya Geological Mapping Project have shown that the late Phanerozoic rift–drift sequence of north-west Australia continues into eastern Indonesia^{10,13}. Accurate dating of the breakup events is possible because of the highly fossiliferous sequence exposed in the Misool Archipelago¹³ and shows that the post-breakup unconformity in Western Irian Jaya is of Upper Liassic (early Jurassic) age (Fig. 2). This is ~10 Myr earlier than in north-west Australia where the post-breakup unconformity is of Bajocian (middle Jurassic) age¹⁵. The earlier occurrence of breakup in western Irian Jaya is consistent with the southward younging of breakup events along the western margin of the Australian continent (Fig. 3), and suggests that the rift–drift sequence in western Irian Jaya formed as a

Fig. 1 Distribution of Gondwana microcontinents around the Banda Sea.

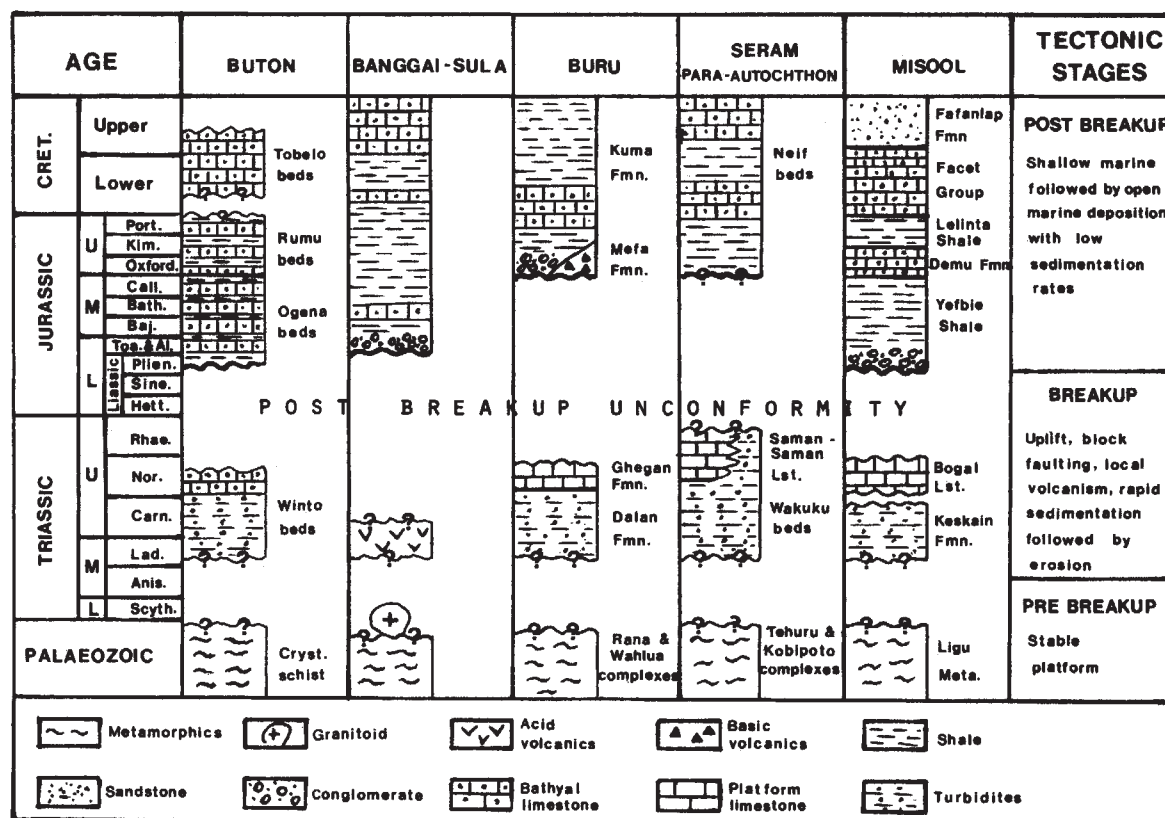
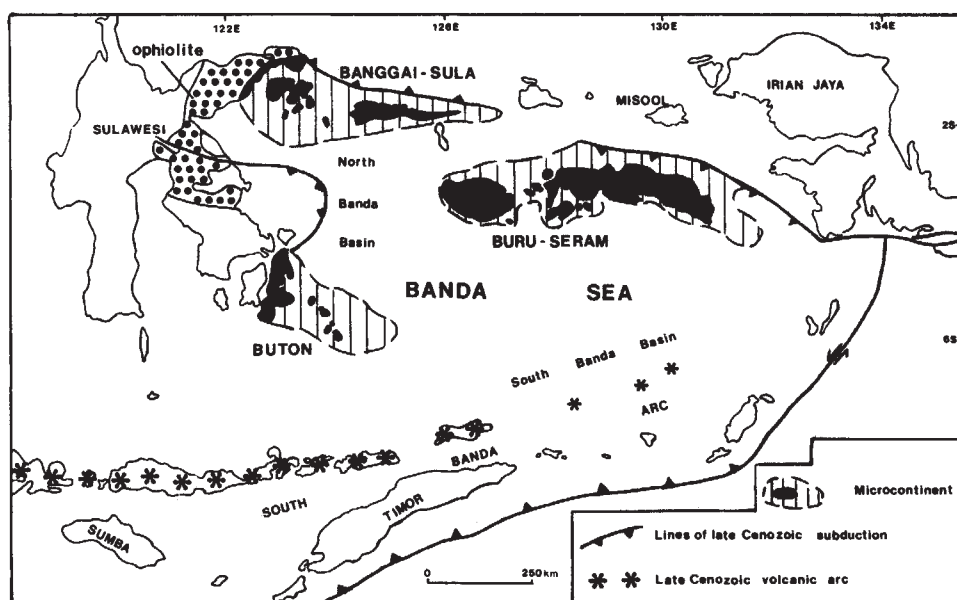


Fig. 2 Late Palaeozoic and Mesozoic of Buton¹⁶, Banggai-Sula^{16,27}, Buru-Seram²⁷⁻²⁹ and Misool¹³, in terms of the tectonic stages of a rift-drift sequence.

consequence of the breakup of Gondwana which led to the opening of the Indian Ocean.

By comparing the Palaeozoic and Mesozoic sequences of the Buton, Banggai-Sula (Sula Spur) and Buru-Seram microcontinents from the north and west margins of the Banda Sea with that of the Misool Archipelago it is possible to interpret the geological history of each microcontinent in terms of the tectonic stages of a rift-drift sequence (Fig. 2). The north Banda Basin has the Banggai-Sula microcontinent to the north and the Buton microcontinent to the south; to the north-west it is bordered by the Jurassic-Cretaceous ophiolite complex of

eastern Sulawesi¹⁶, while to the south-west it is separated from eastern Sulawesi by a late Cenozoic subduction zone¹⁷ (Fig. 1).

The post-breakup unconformity, which is correlated with the onset of spreading in a new ocean basin, is of Upper Liassic (late early Jurassic) age on both Buton and Banggai-Sula microcontinents (Fig. 2). Although the Buton microcontinent has collided with the south-east arm of Sulawesi¹⁸ there is no evidence for transcurrent faulting or subduction along the northern and southern margins which appear to represent passive continental margin so that the adjacent sea floor should be of Jurassic age. The Banggai-Sula microcontinent is usually

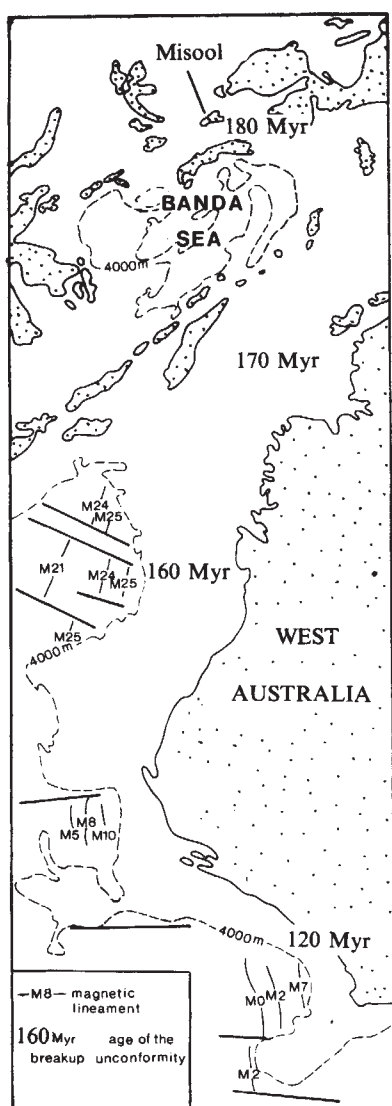


Fig. 3 Margin of the west Australian continent showing the southward younging of the breakup unconformity within the rift-drift sequence. Ages are from refs. 15, 25, 26. Magnetic lineaments and bathymetry are from ref. 17.

interpreted as a fragment, enclosed by transcurrent faults, which was detached from New Guinea some time in the Cenozoic^{18,19}. This interpretation has been questioned by Silver²⁰ and the recognition of a rift-drift sequence with northern Australian affinities on Banggai-Sula (Fig. 2) suggests that it separated from Gondwana by rifting during the early Jurassic (and not by later transcurrent faulting). The southern margin of this microcontinent may also be a passive margin, and the adjacent oceanic crust Jurassic in age.

The evidence from these two microcontinents suggests that the north Banda Basin is floored by Jurassic oceanic crust which is consistent with the age of the east Sulawesi ophiolite which is probably obducted Banda sea floor.

The Buru-Seram microcontinent, which borders the south Banda Basin along its northern margin, has a post-breakup unconformity of Oxfordian (early late Jurassic) age. Although subduction has occurred along the northern margin of this microcontinent^{18,20}, there is no evidence for subduction or transcurrent faulting along the southern margin so that it appears to represent a passive continental margin and the adjacent sea floor should also be of Jurassic age.

An estimate of the age of the sea floor along the southern margin of the south Banda Basin can be obtained by reconstructing this margin before the late Cenozoic subduction which

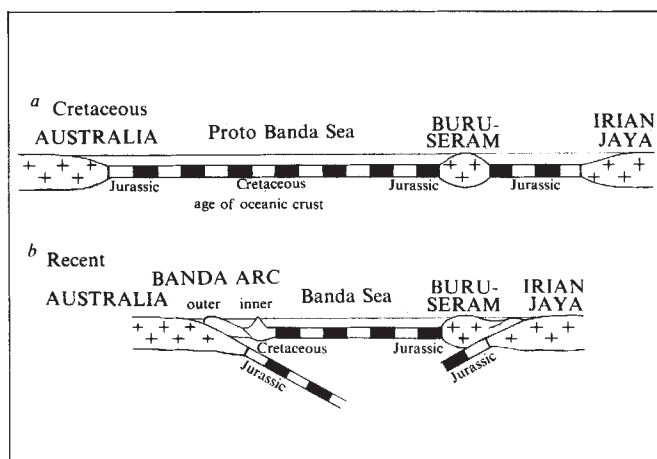


Fig. 4 The South Banda Basin showing how Cretaceous oceanic crust was trapped beneath the Inner Banda Arc.

has occurred in the region (Fig. 4). All the tectonic models of the southern Banda Arc agree that subduction has occurred along a north-facing subduction zone located at some point to the south of the inner or volcanic arc and that the volcanic arc is constructed on oceanic crust^{2,21-24}. Consumption of 800 km (ref. 20) of Indian Ocean sea floor along this subduction zone would destroy all the middle to late Jurassic sea floor that had formed off north-west Australia and leave early Cretaceous sea floor to the north of the outer arc (Fig. 4b).

This analysis suggests that the Banda Sea floor should be of Jurassic age in the north and Cretaceous age in the south. It is not possible, at this stage, to ascertain whether this hypothetical simple younging from north to south is real because the location of the former spreading centre is unknown. If it was to the south of the southern Banda Arc and has been subducted, then the simple southerly younging pattern may be correct and is consistent with the spreading rate for the sea floor of the Argo Abyssal plain¹⁷ off north-west Australia. However, if it is preserved in the Banda Sea region then some symmetrical distribution of ages across it could be expected.

The evidence from the geology of the microcontinents along the northern and western margins of the Banda Sea strongly suggests that the Banda Sea is floored by oceanic crust of late Jurassic to early Cretaceous age which is consistent with the water depths, heat flow and sediment thickness. The age of the onset of spreading suggests that it is related to the opening of the Indian Ocean and supports the suggestion of Bowin *et al.*⁴ that the Banda Sea is trapped Indian Ocean floor.

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Photoreduction of manganese oxides in seawater and its geochemical and biological implications

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Manganese is an essential micronutrient for all organisms. Its requirement by plants is particularly high because of its role in the oxidation of water in photosynthesis^{1–5}. According to thermodynamic considerations, manganese should exist in oxic waters as MnO_2 (ref. 6) which is insoluble and, therefore, not directly available for plant nutrition. In contrast to thermodynamic predictions, however, most of the manganese in near surface seawater exists as soluble reduced Mn(II) (ref. 7). Although slow oxidation kinetics are at least partially responsible for the presence of Mn(II) in oxic waters^{8,9}, reduced manganese, nevertheless, should be converted to particulate manganese oxides (at rates that depend on several kinetic factors^{10,11}) and be lost from the water column by sinking¹². We report here experiments that demonstrate photoreduction of manganese oxides by dissolved organic substances (humic substances) in seawater. Such reactions appear to be important in maintaining manganese in a dissolved reduced form in photic waters, thereby enhancing its supply to phytoplankton.

Reduction of manganese oxide was investigated by measuring the dissolution of ^{54}Mn oxides prepared from $^{54}\text{MnCl}_2$ by permanganate oxidation¹³: oxides prepared by this procedure¹³ have an approximate composition of $\text{MnO}_{1.8}$. Such mixed oxidation state oxides (referred to here as MnO_x) are characteristic of those found in natural waters¹⁴. Experiments were conducted in coastal seawater, and in offshore seawater with and without additions of marine humic acids. Coastal water was collected near the mouth of the Newport River estuary, North Carolina, and was used within 2 h. Offshore near-surface seawater was collected¹⁵ from the Gulf Stream off the North Carolina coast and was stored in the dark for either one week or two months before use. Marine humic acid (MHA) was isolated with Amberlite XAD-2 resin from sea-water collected (5 m depth) 110 km south of Cape San Blas, Florida^{16,17}.

Experiments were initiated by pipetting a suspension of $^{54}\text{MnO}_x$ into 200-ml water samples to a final concentration of $10^{-8} \text{ mol l}^{-1}$. Samples in 250-ml Pyrex glass bottles were illuminated with either sunlight attenuated to different intensities or light from a bank of fluorescent bulbs. Samples of 10–20 ml were withdrawn hourly and filtered through 0.2 μm pore size Nuclepore filters, which quantitatively retained the MnO_x . Filtrates were measured for ^{54}Mn by γ -ray scintillation counting. The percentage of MnO_x dissolution was computed from the amount of ^{54}Mn in the filtrate relative to that added to the samples.

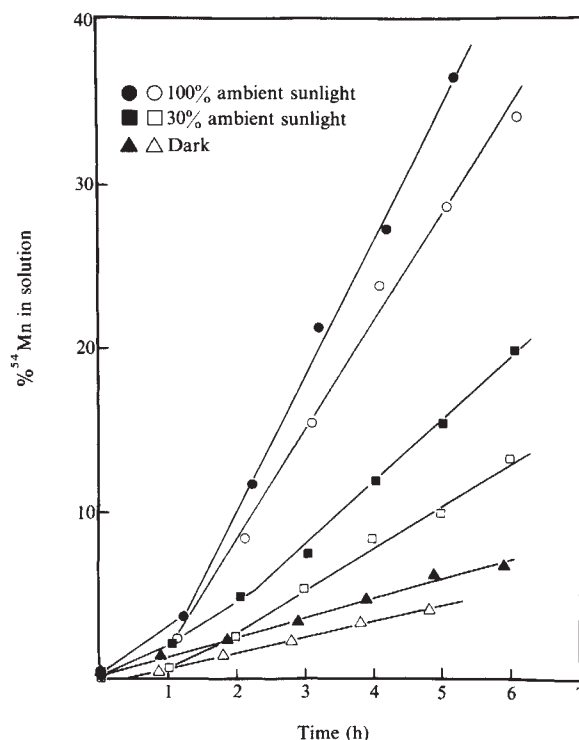


Fig. 1 Dissolution of $^{54}\text{MnO}_x$ in 35‰ coastal seawater at pH 8.1 and 21 °C. Samples were illuminated with ambient sunlight on a partially cloudy day ($500\text{--}2,000 \mu\text{E m}^{-2} \text{ s}^{-1}$) and with sunlight attenuated to 30% with neutral density screens. Bottles were wrapped in aluminum foil for dark subsamples. Closed and open symbols are for subsamples with and without $1.0 \mu\text{mol l}^{-1} \text{ MnCl}_2$ respectively.

At least some MnO_x dissolution occurred with time in all samples. Rates of dissolution increased considerably with increasing light intensity (Figs 1–4) and with the addition of MHA (Figs 3, 4). Dissolution was a linear function of time in the dark provided the samples contained $1 \mu\text{mol l}^{-1} \text{ MnCl}_2$, which was added to displace newly formed $^{54}\text{Mn}^{2+}$ from adsorption sites on MnO_x (ref. 14). Because of this displacement, the amount of ^{54}Mn in solution with time was higher in samples containing added MnCl_2 (Fig. 1). Rates of $^{54}\text{MnO}_x$ dissolution increased with time for samples exposed to light, but approached constant rates after 1–4 h depending on the sample, the light source and intensity, and whether or not MnCl_2 had been added. The final rates of $^{54}\text{MnO}_x$ dissolution in coastal seawater samples exposed to full sunlight were about 7–8 times higher than rates for samples in the dark. Similar rates were observed in filtered (0.2 μm pore size Nuclepore) and unfiltered coastal seawater in preliminary experiments, indicating that dissolved rather than particulate substances are responsible for MnO_x dissolution.

Since manganese oxides are insoluble, their photo-enhanced dissolution must proceed through a change in oxidation state. Both reduced manganous ions, Mn^{2+} , or permanganate ions, MnO_4^- , are highly soluble, but the presence of oxidizing agents sufficiently strong to oxidize MnO_x to MnO_4^- is unlikely in seawater. On the other hand, seawater contains a wide spectrum of dissolved organic compounds¹⁸, many of which are reasonably strong reducing agents: several organic compounds including ascorbic acid, gallic acid, and tannic acid reduce MnO_x to soluble Mn^{2+} (refs 14, 19). We have demonstrated that a major class of organic compounds in seawater, humic acids, greatly enhance MnO_x dissolution. Similarly, in other experiments, we found that humic and fulvic acids present in river water also bring about MnO_x dissolution and that this reaction is also strongly stimulated by light.

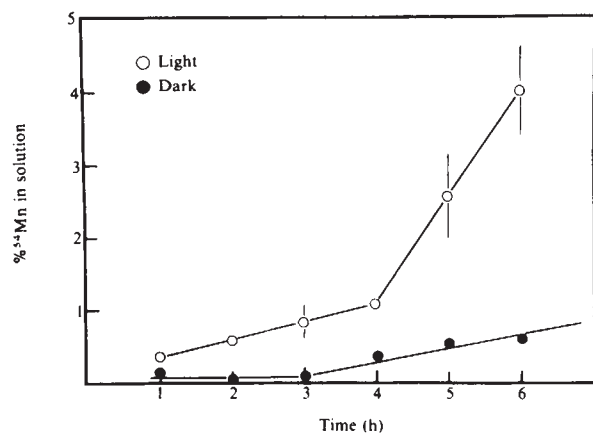


Fig. 2 Dissolution of $^{54}\text{MnO}_x$ in 36‰, pH 8.2 Gulf Stream seawater stored in a 50 l polyethylene carboy in the dark at 4 °C for 1 week. Illumination was provided by natural sunlight ($2,000 \mu\text{E m}^{-2} \text{s}^{-1}$) on a sunny day. Experimental temperature was 18 °C. Error bars indicate the range for two replicates.

From the above evidence, it seems that the dissolution of MnO_x in seawater results from its reduction to Mn^{2+} by organic compounds such as humic acids. Enhancement of this dissolution by light is apparently due to the photoactivation of organic compounds that are either dissolved or adsorbed onto MnO_x surfaces. Photoactivated reduction of other transition metals, Cu^{2+} and Fe^{3+} , by carboxylamine ligands is well documented^{20,21}. Of more direct relevance here, however, is the observation that iron is reduced from the ferric to the ferrous state by natural humic substances²². This reaction, like the dissolution of MnO_x , is strongly stimulated by light.

Light activated dissolution of manganese oxides can at least partially explain the unusual vertical distributional pattern of manganese in the ocean. Unlike other trace metals (such as Cu, Zn, Cd, and Ni) whose concentrations increase with depth due to biological scavenging at the surface and regeneration at depth²², manganese profiles usually show a pronounced maximum in the photic zone²³⁻²⁶. For example, manganese concentrations in ocean waters of the California Current decrease by 5–10-fold in the depth interval 50–100 m (ref. 7). Furthermore, only ~1% of the manganese in the upper 25 m is in a particulate form. As dissolved manganese concentrations decrease sharply near the bottom of the photic zone, particulate concentrations increase to values of up to 30% of the total manganese.

We suggest that the surface maxima in manganese concentrations in the sea are due to two important factors: (1) high input rates to surface waters from continental sources via aeolian and riverine transport²⁷; and (2) photoactivated reduction of manganese oxides to Mn^{2+} . The importance of the latter process would be to dissolve manganese oxides entering the surface of the ocean (for example, as oxide coatings on minerals) and to prevent appreciable oxidation of dissolved Mn^{2+} to particulate manganese oxides which, through sinking, would be lost from the water column. In surface waters there is an abundance of light and a relative abundance of dissolved organic matter derived directly or indirectly from photosynthesis. In these waters, photoactivated reduction by dissolved organic compounds would redissolve newly formed manganese oxides, explaining the overwhelming predominance of dissolved over particulate manganese in surface seawater. Due to decreases in both light and dissolved organic matter with depth, manganese oxides formed in seawater below the photic zone would be far less likely to be redissolved by reduction, and the formation of such oxides would explain the increase in particulate manganese near the bottom of the photic zone. The sinking of these particles, in turn, would appreciably contribute to the sharp decrease in manganese concentrations with depth below the photic zone.

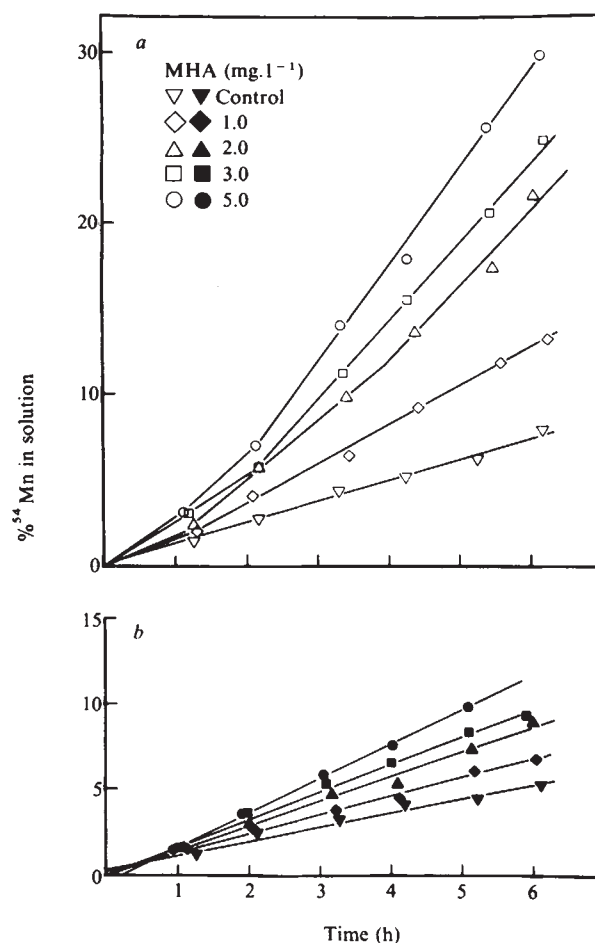


Fig. 3 Effect of marine humic acids (MHA) on the dissolution of $^{54}\text{MnO}_x$ in 2-month old Sargasso seawater at 20 °C and pH 8.1. All samples contained $1.0 \mu\text{mol l}^{-1}$ MnCl_2 to help facilitate desorption of reduced $^{54}\text{Mn}^{2+}$. *a*, Open symbols are for samples exposed to light ($290 \mu\text{E m}^{-2} \text{s}^{-1}$) from fluorescent bulbs (Vita-Lite, Duro-test Corp.). *b*, Closed symbols are for parallel samples maintained in the dark.

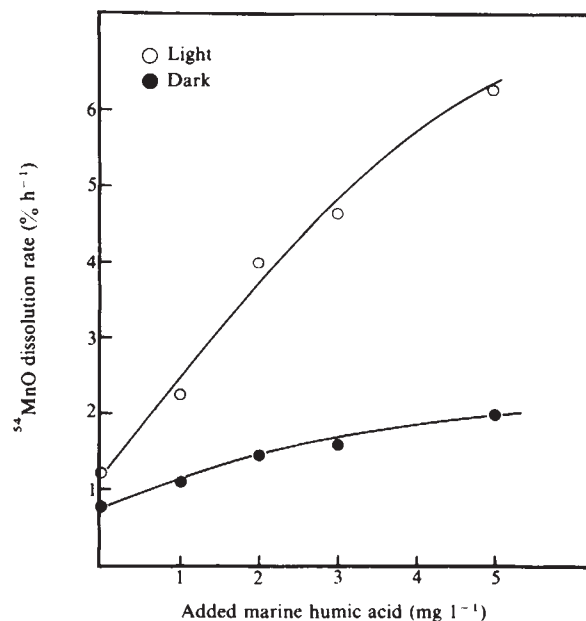


Fig. 4 Effect of MHA addition on the final linear rates of $^{54}\text{MnO}_x$ dissolution in Sargasso seawater. Rates were determined from data plotted in Fig. 3. Rates for samples exposed to light are for the time interval 2–6 h. Those for dark samples are for the interval 1–6 h.

Because of its requirement in photosynthesis, the availability of manganese in surface waters can have considerable biological importance. For example, deep seawater that is upwelled to the surface often contains insufficient manganese to support maximum plant growth²⁸⁻³⁰. From the above discussion, it appears that photoreduction of manganese oxides by dissolved organic compounds is an important process in the solubilization and retention of manganese in the photic zone, thereby, helping to maintain an adequate supply of manganese to phytoplankton. Since the ultimate source of the organic reducing agents (such as humic acids) is the biological community itself, the production of such compounds can be viewed as a biological conditioning

of surface seawater which helps facilitate maximum primary productivity in the sea. More specifically, the photochemical reduction of manganese oxides by those compounds appears to constitute a set of external photochemical reactions that enhances the supply of manganese required in the internal photochemical reactions of photosynthesis.

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²³⁰Th/²³⁴U age of a Mousterian site in France

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Pech de l'Azé is a group of caves and rock shelters near Sarlat, in the Dordogne district of south-west France. In the cave Pech I there occurs a remnant of breccia containing Mousterian (middle Palaeolithic) artefacts of Denticulate and Typical varieties, overlying a travertine layer. We have used the ²³⁰Th/²³⁴U dating method to determine the age of this travertine: 123 ± 15 kyr. This age is consistent with deposition of this travertine during isotope state 5e, the warmest substage of the last interglacial. We show here that this result is consistent with geological and palaeontological estimates of the age of sediments filling the cave¹.

Bordes² described a stratigraphical sequence in Pech I and II containing artefacts of the Acheulian and Mousterian traditions. The latter industry was limited to beds which he determined to have been deposited just after the last interglacial stage, and which he referred to as the Riss/Würm (R/W). Layers of stalagmitic calcite which occur intercalated with sediments at this site can be dated by the uranium-series methods³, because at the time of deposition they commonly contain traces of uranium but are relatively free of thorium. The stalagmites at Pech de l'Azé contain very low concentrations of U (<0.1 p.p.m.) which makes accurate dating difficult, but their purity and texture appear satisfactory for dating purposes. Previous studies of the sediment layers^{1,2} have given their ages in terms of the classical Alpine glacial sequence (Riss, Würm and so on) for which various absolute chronologies have been suggested⁴. We shall use the isotopic record and chronology of deep-sea sediments⁵ to infer the climatic significance of the absolute ages which we have obtained.

The samples of calcite were analysed using methods described by Gascoyne *et al.*⁶. Activity ratios of ²³⁰Th/²³⁴U and ²³⁴U/²³⁸U were determined by α -particle spectroscopy. For those samples with a ²³⁰Th/²³²Th ratio of <20, a correction was made for common ²³⁰Th, assuming that the initial value of the ratio had been 1.25 (see ref. 3).

Bordes² recognized several variants of the Mousterian tradition in these shelters: in Pech II a sequence including Typical, Denticulate and Ferrassie types; in Pech I, Mousterian of Acheulian tradition, types A and B. The sediments of Pech I were deposited after those of Pech II following a period of fluvial erosion which washed out a pre-existing mass of sediments coeval with those of Pech II (ref. 2). Two residual masses of these sediments, now calcite-cemented breccia, adhere to the wall of Pech I. They contain artefacts that resemble those of certain beds of Pech II. This breccia was deposited at the end of the last interglacial, and was largely eroded away during the 'Würm I-Würm II' interstadial¹.

The outer of the two breccia masses ('Breccia I' in ref. 2) contained a pure calcite flowstone layer near its base, deposited on a coarse breccia cemented with calcite (Fig. 1). Above the flowstone are layers of impure travertine, calcite cemented sand, and finally a breccia, which makes up the main volume of the mass (see Fig. 6 in ref. 2). Another mass of travertine embedded in calcite-cemented sediment, found closer to the mouth of the cave, was believed to have been deposited on the wall of the cave earlier than Breccia I (F. A. Bordes, personal communication). However, it appears to have been deposited on top of the detrital layers, as stalactites filling a solutional cavity. The host sediment itself was lacking in artefacts or faunal remains.

The breccia underlying the flowstone layer in Breccia I, while lacking artefacts, contained the bones of a cave bear, possibly *Ursus deningeri* (F. A. Bordes, personal communication). Above the flowstone, and throughout the correlative Breccia II were found small numbers of Mousterian artefacts of Denticulate and Typical varieties. Pollen from below the travertine of Breccia I "give indications of a very cold climate"², while higher layers correspond to warmer climate, with from 21 to 35% tree pollen. Both the artefact assemblage and the pollen suggested that these breccias were equivalent in age to layer 4 of Pech II, which had been attributed to the 'early Würm' (Würm I). A further, loose breccia block in Pech I contained

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Table 1 Radiometric data

Sample	Horizon	$^{234}\text{U}/^{238}\text{U}$	$^{230}\text{Th}/^{234}\text{U}$	$^{230}\text{Th}/^{232}\text{Th}$	[U] (p.p.m.)	Age (kyr)	Average age (kyr)
Pech I							
PA.5	Outer travertine	2.087±0.351	0.639±0.078	>1,000	0.04	99±18	123±15
PA.8-1	Breccia I	1.248±0.141	0.726±0.065	24±8	0.09	128±20	
PA.8-3	Breccia I	1.302±0.083	0.648±0.183	>1,000	0.10	108 ⁺⁶⁶ ₋₄₂	
PA.9	Breccia I	1.274±0.127	0.760±0.069	10±3	0.05	135 ^{+32*} ₋₃₆	
PA.11-1	Breccia I	1.177±0.216	0.770±0.112	9±4	0.09	136 ^{+60*} ₋₄₃	
PA.11-2	Breccia I	1.281±0.094	0.668±0.062	11±7	0.09	106±23	
Pech II							
PA.13	Bed 3	1.208±0.110	0.729±0.065	3.5±0.3	0.06	103 ^{+30*} ₋₂₅	(240±30†)
PA.14 A-1	Bed 5	1.309±0.233	0.948±0.158	1±0.1	0.02	>350	
PA.14 A-2	Bed 5	1.056±0.092	1.060±0.096	1±0.1	0.06	202 ^{+(>350)} ₋₂₅₀	
PA.14 C	Bed 5	1.101±0.182	0.846±0.245	4±2	0.10	160 ^{+(>350)} ₋₁₃₀	
PA.14 C	Bed 5	1.025±0.047	1.013±0.049	6±0.4	0.08	>350	
(Averages for PA.14:		1.123±0.128	0.967±0.092)				

* Corrected for common ^{230}Th assuming initial $^{230}\text{Th}/^{232}\text{Th} = 1.25$.

† Calculated from average $^{230}\text{Th}/^{234}\text{U}$ obtained by graphical analysis (see text).

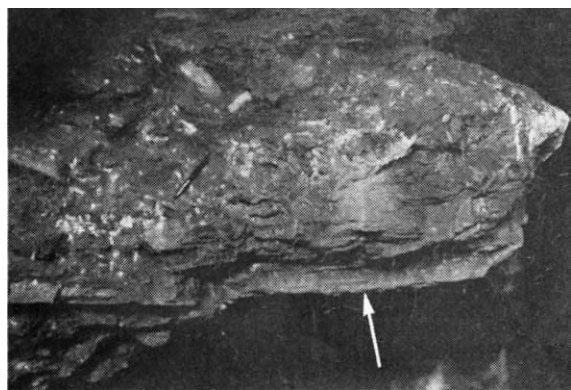


Fig. 1 Outer breccia ('I'), showing flowstone layer near base (arrow). Breccia above flowstone contains Mousterian artefacts. Underlying breccia contains bones of *Ursus deningeri*.

a Mousterian with hand-axes, suggesting the presence of Mousterian of Acheulian tradition before the fluvial scouring out of the breccia in early Würm².

The inner breccia mass gives an average Th/U age of 123 ± 15 kyr (Table 1). This date is consistent with deposition of the stalagmitic layer in isotope stage 5e, the age of which is estimated to be 128 kyr (ref. 5). The error in the age determined here would also permit deposition as recently as 105 kyr, corresponding to isotope stage 5c, another warm stage. The outer breccia gives an age of 99 ± 18 kyr which cannot be resolved statistically from the age of the inner breccia. Thus both dates indicate a period of stalagmite deposition sometime within the first half of isotope stage 5.

Studies elsewhere have indicated that speleothem deposition is most active during interglacial stages⁷. Hence the flowstone layer probably represents either stage 5e or 5c. The pollen record for the post-flowstone layers of Breccia I has been correlated with that of layers 4C and 4B of Pech II, which in turn overlie cryoclastic sediments of layer 5, interpreted by Laville¹ to represent the beginning of the Würm glacial. We could reconcile our absolute dates with this correlation by assigning the deposition of the flowstone to stage 5c and attributing the cold flora of the underlying breccia to stage 5d. However, our data would also allow isotope-stages 5e and d to correlate respectively with the flowstone and overlying breccia of Breccia I. Then the cryoclastic deposits of layer 5 (Pech II) would represent isotope stage 6, or 'latest Riss' in Laville's

terminology. No true soil is found below layer 5, as might be expected to form during a climatic optimum². Also, layer 5 is distinctly richer in signs of cryoclastic activity than layers above or below it, consistent with formation during the coldest part of the penultimate glacial. The base of Breccia I (Pech I) would also correlate with the glacial maximum at the end of isotope stage 6. This is consistent with the pollen record from it, as noted above. If this chronology is correct, some signs of an interglacial stage should be evident in the sediments of layer 4D, Pech II, although none have yet been described.

The calcite sample PA 13 appears to have been precipitated *in situ* in Bed 3 of Pech II. The large error in its date is due to the low U content and poor yield in extraction of U and Th. The age (103 ± 27 kyr) is consistent with deposition early in the last glacial ('in the third climatic phase of Würm I' as described by Laville¹). The large error limits would, however, also permit deposition of PA13 during stage 5e, contemporaneous with the flowstone of Breccia I.

Samples PA 14 A and C are stalactitic slabs originally deposited on the roof of Pech II and subsequently fallen into layer 5. The age of such deposits can only give a maximum limit on the time of deposition of layer 5. Their estimated age of 240 kyr is based on a calculated mean value for their $^{230}\text{Th}/^{234}\text{U}$ ratio, using a plot of $^{230}\text{Th}/^{232}\text{Th}$ against $^{234}\text{U}/^{232}\text{Th}$. The age suggests that the roof of the cave was actively accumulating stalactitic coatings during interglacial isotope stage 7. While the age of layer 5 is given as 'early Würm'¹, which we can interpret as post-stage 5e, we have already noted a possible correlation with the end of stage 6. Indeed, it seems quite likely that stalactitic coatings which had formed during previous interglacial (stage 7) might have been dislodged together with many blocks of limestone, by intense cryoclastic action in the coldest phase of stage 6 (late Riss).

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The tibio-fibular articulation in *Apidium phiomense*, an Oligocene anthropoid

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***Apidium phiomense*, a primitive anthropoid from the Oligocene of Egypt, shows partial fusion of the distal tibio-fibular articulation with extensive apposition of the two bones, a unique condition among higher primates. Functionally, this condition restricts the tibio-talar joint to simple hinge movements which, together with other features in the skeletal anatomy of *A. phiomense*, suggests that this species was a leaper. The condition in *Apidium* suggests phenetic and possible phylogenetic links with platyrrhine monkeys, many of which show similar, but less extensive fibular apposition.**

The distal articulation between the tibia and fibula shows considerable variation among living mammals, much of which can be related to differences in locomotor adaptations^{1,2}. In a review of this joint, Barnett and Napier² recognized three types of articulation. (1) An immobile fibula, either wholly or partially fused to the tibia, is found among members of many mammalian orders. In swimming and burrowing animals (for example, sea lions, *Otaria californiana*, and the cape golden mole, *Chrysochloris asiatica*) the stout fibula is fused to the tibia both proximally and distally. In saltatory species (for example, rabbits, *Oryctolagus cuniculus*; jerboas, *Jaculus jaculus*; and tarsiers, *Tarsius* spp.) a slender flexible fibula joins the tibia by a synovial joint proximally and is fused distally by a synostosis. The tarsier, a small Asian prosimian, is the only living primate in which Barnett and Napier² recognized any type of fibular fusion. (2) A mobile fibula, joined to the tibia by a synovial joint both proximally and distally, is characteristic of most primates and is also found in bears and cats. This type of fibula allows a variety of rotational movements at the ankle joint in addition to flexion and extension, and has been related to movement on uneven and arboreal substrates. (3) Finally, Barnett and Napier² identified a group of mammals with an 'intermediate fibula' joined proximally by a synovial joint and distally by a syndesmosis, often associated with a small synovial recess which is found in a variety of noncursorial ungulates (for example, pigs and tapirs) as well as canids, rodents, and a tree shrew (*Tupaia belangeri*). In experimental tests, they found no evidence of rotatory movements at the distal talar joint in dogs or pigs, but argued that this latter group generally showed no extreme locomotor specializations.

Two groups of fossil primates are known from the Oligocene of Egypt³. The larger hominoids (*Aegyptopithecus* and *Propliopithecus*) have been widely recognized as the ancestors of all later apes, and probably Old World monkeys as well³⁻⁶. The smaller parapihithecids (*Parapihithecus* and *Apidium*) have been variously identified from dental evidence as (1) the ancestors of Old World monkeys⁷, (2) a specialized side-branch of early catarrhines⁶, or (3) a primitive group of anthropoids ancestral to New World monkeys⁸. Skeletal remains of *Apidium phiomense* were first described by Conroy⁹ who noted that the parallel-sided talus of this species allowed very little rotational movement at the tibio-talar joint. Recent Fayum expeditions have yielded many new postcranial elements of these early anthropoids, including a complete, articulated ankle of *Apidium phiomense*. The specimen, DPC 1303 (Fig. 1a), was collected in Quarry I in 1980 by Richard F. Kay and includes the distal

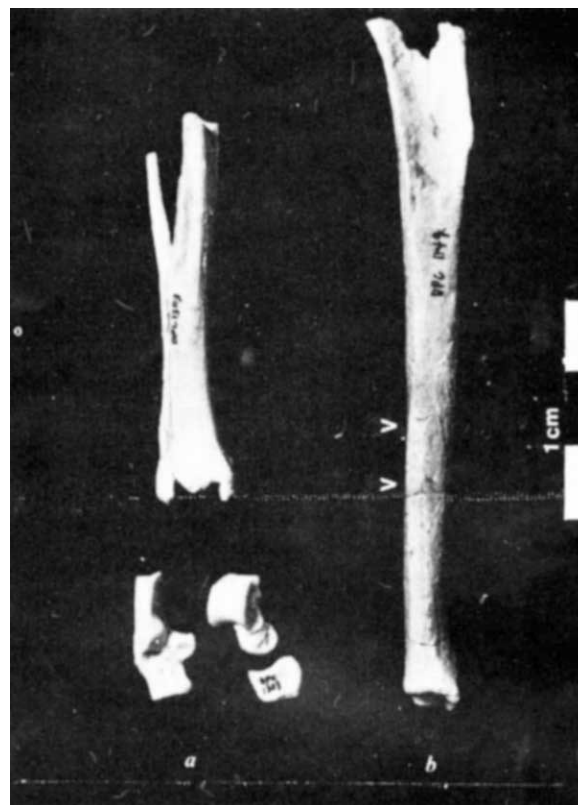


Fig. 1 New limb bones of *Apidium phiomense*. a, DPC 1303, associated right partial tibia and fibula, calcaneus, talus and navicular. b, DPC 1149, nearly complete right tibia showing bony scar where fibula articulated (arrows).

two-thirds of the tibia and fibula, talus, calcaneus and navicular from a single sub-adult individual.

The talus and calcaneus of the new specimen are identical with specimens described previously⁹ and assigned to *Apidium phiomense* on the basis of size regressions, abundance of this taxon in the fauna, and concordance with other skeletal elements assigned to this species. The most notable features of the talus are the long medially deviated neck, indicative of a grasping hallux; and prominent, subequal, parallel-sided trochlear ridges indicative of a hinge-like tibio-talar joint with little rotatory mobility. The former feature is a characteristic of all living primates while the latter is most prominently developed in leapers and cursorial species of many orders. The calcaneus shows a pronounced peroneal tubercle laterally and a deep groove for flexor hallucis longus on the inferior surface of the sustentaculum. These two features are characteristic of all living primates and are poorly developed or absent in other mammals⁹. The navicular is similar to the same bone in small cebids (for example, *Saimiri*).

The most striking, and surprising, feature of this specimen is the extensive apposition and apparent fusion of the tibia and fibula for 31 mm proximal to the distal articulation, or approximately 40% up the shaft. In contrast with *Tarsius*, the two bones in *Apidium* are not synostosed or fused, but remain distinct throughout their length, resembling Carlton's¹ description of the red kangaroo (*Macropus*), "though there is not actual fusion, the tibia and fibula are so moulded together in their lower halves as to form what appears to be one cylindrical bone with a crack in it". The lack of actual fusion but presence of extensive apposition is also verified by numerous other isolated tibiae of *Apidium phiomense* from which the fibula has been separated, leaving a distinct scar on the lateral side of the shaft (Fig. 1b, arrows). In the classification of Barnett and Napier², the tibio-fibular joint in *Apidium phiomense* would be an

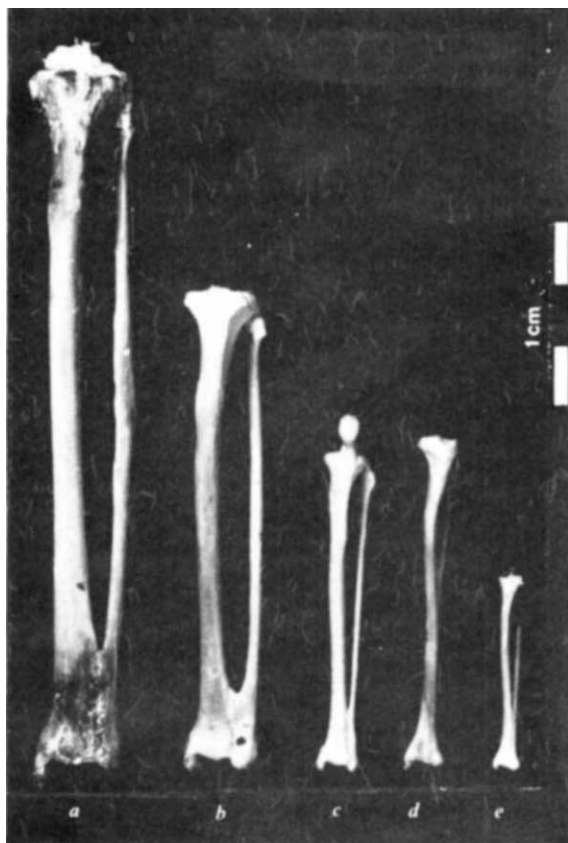


Fig. 2 Limbs of various extant primate species showing extensive apposition or partial synostosis of the tibia and fibula. *a*, *Pithecia pithecia*; *b*, *Saimiri sciureus*; *c*, *Callithrix jacchus*; *d*, *Tarsius syrichta*; *e*, *Microcebus murinus*.

extreme case of type three, 'the intermediate fibula', that approximates a fused condition.

Functionally, this extensive distal apposition and syndesmosis of the leg bones limits or even eliminates rotational movements at the tibio-talar joint, restricting this joint to simple flexion-extension movements in a sagittal plane. The specialized nature of the ankle in *Apidium phiomense* suggests that this species was primarily adapted for leaping, as do many other aspects of its postcranial skeleton, including a long laterally compressed tibia with very proximal insertion of the semitendinosus and gracilis muscles, deep femoral condyles with a pronounced lateral lip of the patellar groove, a stout femoral neck oriented perpendicular to the femoral shaft, a long ischium, and a low intermembral index.

The extensive tibio-fibular syndesmosis in *Apidium phiomense* is unique among higher primates. As Barnett and Napier² observed, *Tarsius* (Fig. 2*d*) is the only living primate in which the tibia and fibula are always totally joined. There is evidence that fossil tarsoids from the Eocene and Oligocene of Europe show a similar fused condition^{6,10}. In light of other evidence suggesting a tarsoid origin for higher primates^{11,12}, it is both tempting and very reasonable to argue that the 'near fusion' of tibia and fibula in *Apidium* is further evidence that these early anthropoids are descended from a tarsoid lineage characterized by tibio-fibular fusion. *Apidium* might reflect either the primitive haplorhine condition or a neotenic 'reversal'¹³ to an unfused condition.

However, examination of the tibio-fibular articulation in a wide range of living primates shows that the form of this articulation is far more diverse and variable than described by Barnett and Napier². For example, the mouse lemur, *Microcebus murinus* (Fig. 2*e*), shows an extensive tibio-fibular syndesmosis very similar to that in *Apidium*. Furthermore, *Microcebus*, tarsiers and all known fossil tarsoids also show tarsal elongation not found in the Oligocene anthropoid. Thus, on the basis of

ankle morphology, *Apidium* is not obviously more 'tarsier-like' than 'lemur-like', and is clearly different from both.

Further comparisons have shown that a more restricted syndesmosis and occasional synostosis of the distal tibio-fibular joint is remarkably common in many small platyrrhine monkeys, including *Pithecia pithecia* (Fig. 2*a*), *Saimiri sciureus* (Fig. 2*b*), *Callithrix jacchus* (Fig. 2*c*) and *Cebuella pygmaea*. All of these species are frequent leapers which share various other skeletal similarities with *Apidium phiomense*. The tendency for partial fusion of the distal tibia and fibula seen in *Apidium* and small platyrrhines is not present in the Fayum hominoid *Propliopithecus*¹⁴ or any other fossil or Recent catarrhine. The phyletic significance of this and other morphological similarities between *Apidium* and living platyrrhine^{9,14,15} is unclear at present and there are several possible interpretations. One possibility is that an extensive syndesmosis and 'incipient fusion' of the tibia and fibula is the primitive anthropoid (or haplorhine) condition. The similarity between *Apidium* and small platyrrhines would then be primitive similarities of no phyletic significance while the 'separate' tibia and fibula of all other catarrhines and most large ceboids would be an advanced condition. Alternatively, if the separate tibia and fibula found in *Propliopithecus* and all later catarrhines is the primitive anthropoid condition, the 'incipient fusion' seen in *Apidium* and many small platyrrhines could be interpreted as either a unique specialization (synapomorphy) linking *Apidium* with New World monkeys or a functional specialization which has evolved independently in each of several primate groups with no phyletic significance. Clearly this feature has evolved independently in many other mammal lineages. This history and phyletic significance of this character for early anthropoid evolution can not be resolved without further information about the same region in Eocene prosimians and in the earliest platyrrhines.

Nevertheless, this new material reinforces two conclusions that are increasingly obvious from consideration of the skeletal anatomy of Fayum anthropoids. First, these extinct higher primates frequently show unique combinations of morphological features that are not found within any single living higher primate species. Second, where similarities exist they are more frequently to living and fossil platyrrhines or to prosimians than to living catarrhines^{5,9,14-16}. Thus in most aspects of skeletal morphology, but not dental morphology^{6,12}, *Apidium* and, to a lesser extent, the larger Fayum hominoids show many skeletal similarities that would make them suitable ancestors for platyrrhine monkeys. Conversely, living platyrrhines appear to retain a skeletal anatomy very similar to that found in Oligocene primates from Africa while living catarrhines show many advanced specializations not found among these earliest anthropoids.

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The relationship between density and mean frond weight in monospecific seaweed stands

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The equation

$$w = Kd^{-3/2} (\log_{10} w = \log_{10} K - 1.5 \log_{10} d) \quad (1)$$

defines a boundary condition known as the $-3/2$ power law¹, above which combinations of mean plant weight and density may not occur. In this equation w is mean above-ground plant dry weight, d is plant density and K is a constant. For developing cohorts of plants combinations of mean weight and density approach and then move parallel to this boundary along lines for which $\log_{10} K$ is typically between 3.5 and 4.3². Annual shoot populations produced by clonal perennial herbs approach, but do not cross the line where $\log_{10} K = 4.3$ (refs 3, 4). Dense, natural stands of many species with a wide range of growth forms and age distributions lie close to, but below the same line^{2,5}. Virtually all plant populations for which data are available, therefore, fall below the line given by

$$\log_{10} w = 4.3 - 1.5 \log_{10} d \quad (2)$$

We present here evidence suggesting that this line also defines a boundary condition for mean weight and density combinations in monospecific seaweed stands.

Publications on seaweed stands have not often reported both plant weights and densities, and as a result data relevant to this subject are uncommon in the literature. This may well be because within dense populations of seaweeds it is often impossible to identify separate genetic individuals, or genets⁶. To overcome this difficulty we consider algal stands as populations of fronds. The frond is defined here as the portion of thallus arising from a single vertical projection of a holdfast. It is the functional unit within a population and equivalent to the ramet of higher plants in the way in which it is produced.

Figure 1 illustrates several sets of data relating mean frond weight and density in natural seaweed populations. It can be seen that most of the points fall close to or below the line given by equation (2). They include data for the time-courses of growth of developing populations of the annual species *Saccorhiza polyschides*⁷ and *Chordaria flagelliformis*⁸; one of the *C. flagelliformis* populations (A) was at such a low density that density-dependent self-thinning was unlikely to have occurred, but this population still followed a trajectory parallel to the line defined by equation (2). Data for the perennials *Ecklonia radiata* and *Sargassum sinclairii*, the only subtidal species included, are for a number of even-aged stands⁹. The group of points well above the line represents multi-aged populations of *Ascophyllum nodosum* fronds growing under a range of exposures to wave action at a single point in time¹⁰. Frond weight frequency distributions for this species are extremely positively skewed¹⁰ and the arithmetic means are therefore poor descriptors of the populations. However, the distributions are log-symmetrical (but not log-normal), and when the geometric mean weights are plotted the points are brought down below the line. (Many of Gorham's⁵ measurements were collected from populations with positively skewed weight distributions

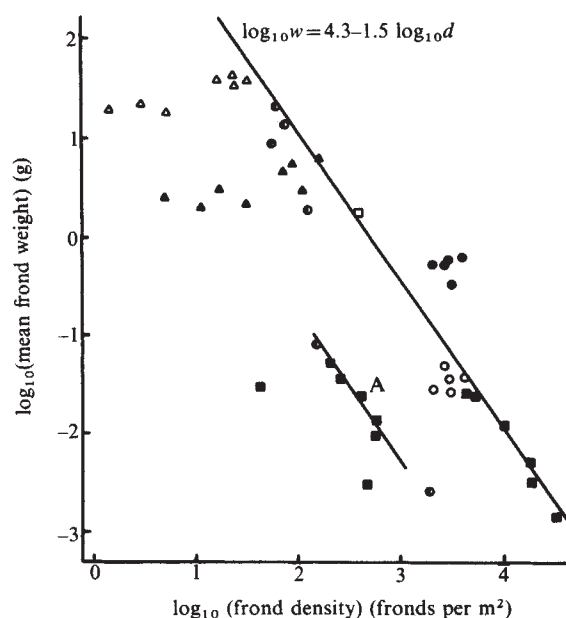


Fig. 1 Relationship between mean frond weight and frond density for natural stands of various marine macrophytic algae growing in monocultures. Data for *Saccorhiza polyschides* and *Chordaria flagelliformis* are for time-courses of growth of stands. Δ , *Ecklonia radiata*⁹. $\blacktriangle$, *Sargassum sinclairii*⁹. $\square$, *Fucus vesiculosus* (southern England, January 1982, unpublished data). $\circ$, *Ascophyllum nodosum* (geometric mean weight)¹⁰. $\bullet$, *Ascophyllum nodosum* (arithmetic mean weight)¹⁰. $\blacksquare$, *Chordaria flagelliformis*⁸. $\circ$, *Saccorhiza polyschides*⁷. Both lines have a gradient of $-3/2$. The upper of the two lines has the equation $\log_{10} w = 4.3 - 1.5 \log_{10} d$.

(personal communication) and it may be that the use of geometric means might have been more appropriate. This would have resulted in some of Gorham's points lying further below the line.)

The position of the boundary condition for combinations of mean weight and density is generally thought to be controlled by the depletion of light passing through canopies¹¹. The amount of light penetrating to a point within a stand will decrease as standing crop and plant height increase. As a consequence the smallest plants will receive a reduced light supply, their growth rates will decrease, and those falling below compensation irradiance will die. A reduction in the overall light received by a stand results in a time-course of development for which the value of $\log_{10} K$ is reduced, although the $-3/2$ gradient does not alter¹²⁻¹⁴. Presumably this is because individuals under reduced light have fallen below compensation irradiance at a lower standing crop, which results in lower densities of surviving plants for a given standing crop. It is unlikely that marine algae will exceed the line defined by equation (2), since during periods of submergence they mostly exist at light levels lower than those occurring at the terrestrial sites for which the equation was derived. They might therefore be expected to have a lower value of $\log_{10} K$. Responses to light supply that are qualitatively similar to the relative sunlight and shade physiological acclimatization of higher plants have been found in preliminary work on at least one seaweed¹⁰. There is therefore little reason to suggest that the same mechanism should not control mean weights and densities in both groups of plants.

If the same boundary constraint does apply to combinations of weight and density of both higher plants and seaweeds, this may have a considerable bearing on the search for a theoretical derivation of the $-3/2$ power law. Because the law would then apply equally to higher plants, with their relatively rigid structures, to the much more flexible seaweeds, whose canopies change in form throughout tidal cycles and to both bushy and linear growth forms, the basis of the law presumably lies at a

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very fundamental level. There should be no need, therefore, to become involved in complex mathematical arguments involving plant shape in order to derive an equation for the limit to combinations of mean plant weight and density¹⁵.

Schiel and Choat recently concluded⁹ that there are fundamental differences between the responses of terrestrial and marine plants to density. It can be seen from Fig. 1 that Schiel and Choat's data fall on or well below the line given by equation (2) and provide no evidence to suggest that the law is violated by the species they have studied. The lack of a gradient of -1.5 through their points is not evidence against the applicability of the law; as both White¹⁵ and Westoby¹⁶ point out, all combinations of density and mean plant weight below the boundary condition of equation (2) are possible. Their data represent populations of different initial densities sampled at a single point in time and not the growth of populations through time; it would not be expected that a regression through such a set of points will give a gradient of -1.5 unless they were all very close to the boundary condition. Most of the data presented by Schiel and Choat are still some way below the greatest mean weights expected for the densities of their stands.

Schiel and Choat's conclusion hinges on the assumption that the only difference between their stands was in plant density. From observing a general increase in plant size and growth rate with increasing density, they concluded that individuals benefit rather than suffer from the close proximity of neighbours. Alternatively, their original assumption of equality of external factors at all stands might not be correct. Although differences in local habitat conditions between stands may not be apparent, differences may still exist, and greatest plant densities and sizes may in fact be occurring in the abiotic conditions most favourable to their species. It is unlikely, for example, that the way in which water currents swirl around the rocks within any boulder field will be uniform and Schiel and Choat's stands were located within such an area. Intertidal fucoids in Eastern Canada may certainly show very large changes in morphology and growth rate over very small distances¹⁷; while the reasons for these changes may not be obvious at low tide, it can often be seen that there are considerable variations in water movement over very short distances as the tide comes in, and these are probably responsible.

Although the responses of higher plants and seaweeds to density may, indeed, differ in some respects as Schiel and Choat suggest, the evidence provided by them is inconclusive, particularly with regard to the $-3/2$ power law. The data presented here indicate that their interpretation is incorrect. Their other proposals could only be confirmed or rejected by growth experiments under strictly controlled conditions, similar to the self-thinning experiments of White and Harper¹⁸, or by following the progress of growth in permanent plots. As such experiments have not been attempted, their proposals remain unproven.

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A subpopulation of rat dorsal root ganglion neurones is catecholaminergic

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The neurotransmitters used by the sensory neurones of the dorsal root ganglia (DRG) are unknown. A proportion of these cells contain physiologically active peptides; for example, subpopulations of small-diameter neurones contain substance P or somatostatin^{1,2}. Although these peptides probably have some influence on synaptic transmission in the dorsal horn of the spinal cord, their status as neurotransmitters is uncertain and it is possible that they coexist with conventional neurotransmitters. In addition, the neurones containing identified peptides account for only a fraction of the DRG sensory neurones^{2,3}. There is evidence that the DRG contain catecholamines within fibres thought to be autonomic, but these substances have not been found within the sensory cell bodies themselves^{4,5}. Moreover, the apparently inappropriate, inhibitory physiological effect of catecholamines in the dorsal horn^{6,7} has argued against their being primary sensory neurotransmitter molecules. We have used here antisera against tyrosine hydroxylase (TH; EC 1.14.16.2) and dopamine- β -hydroxylase (DBH; EC 1.14.17.1), two enzymes specific to catecholaminergic cells⁸⁻¹⁰, to show that a subpopulation of rat DRG neurones is catecholaminergic and that the neurotransmitter they make is probably dopamine. We believe this to be the first report of catecholaminergic sensory neurones.

TH was visualized in the DRG of adult rats using a rabbit anti-TH serum produced and characterized by Joh *et al.*⁹. Sprague-Dawley rats were perfused with 4% paraformaldehyde in saline and the DRG of the lower lumbar region were removed, fixed and mounted as described in Fig. 1 legend. From this tissue, 20- μ m frozen sections were cut on a cryostat at -20°C . The sections were stained with anti-TH (diluted 1:1,000) by the peroxidase-anti-peroxidase method of Sternberger (ref. 11 and Fig. 1 legend). The anti-TH serum labelled a small subpopulation of the sensory neurones of the adult DRG (Fig. 1). The TH-positive (TH⁺) cells were small neurones (diameter $\sim 20\ \mu\text{m}$)¹² and were distributed throughout the ganglion. By examining serial sections of the entire ganglion, we estimated that 1% of the sensory neurones of the L5 ganglia of 3-4-week-old rats were TH⁺. However, there was a striking heterogeneity among the ganglia examined: ganglia L6 and S1 contained considerably fewer TH⁺ neurones ($<0.1\%$) and no TH⁺ cells were observed in ganglia T13 to L4 or in any of the cervical ganglia.

Sections of DRG were also incubated with an anti-DBH serum (diluted 1:1,000) produced and characterized by Lewis *et al.*¹³, but no sensory neurones were labelled with this antiserum. Trivial explanations of this result could be excluded because sympathetic fibres in the ganglion were stained by this antiserum. Furthermore, the anti-DBH serum labelled neuronal cell bodies in frozen sections of rat superior cervical ganglion over a wide range of dilutions. Thus, we conclude that a small proportion of neurones in some rat DRG are TH⁺ and DBH⁺ and are therefore probably dopaminergic.

To confirm that these neurones were catecholaminergic, we sought to demonstrate that they synthesized and stored catecholamine. Previous studies using the paraformaldehyde-induced fluorescence method had failed to show catecholamines in rat DRG neurones⁵ and our initial studies were also negative. However, using the SPG method of De La Torre¹⁴, which we found to be more sensitive, and by treating the rats with colchicine 6 h before death, a small subpopulation of small-diameter sensory neurones were found to be fluorescent (Fig. 2). The size, distribution and number of the fluorescent

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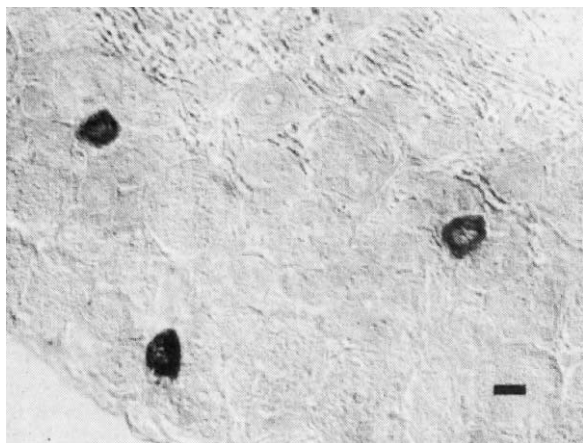


Fig. 1 Light micrograph of a section of 21-day-old rat DRG labelled with anti-TH serum visualized by the peroxidase-anti-peroxidase (PAP) technique of Sternberger *et al.*¹¹. The figure shows three TH⁺ neurones and many unlabelled cells. No staining was observed when normal rabbit serum was used instead of the anti-TH serum. DRG were removed from 21-day-old Sprague-Dawley rats, fixed by overnight immersion in 4% paraformaldehyde in phosphate-buffered saline (PBS), followed by immersion in 5% solution of sucrose in PBS containing 0.05% sodium azide. The ganglia were then mounted in OCT embedding medium (Tissue Tec), frozen in liquid nitrogen and cut on a cryostat at -15°C into 20- μm sections which were melted onto gelatin-coated glass slides. The sections were incubated with 0.3% H_2O_2 in distilled water for 30 min to block endogenous peroxidase activity and were incubated overnight in a humid atmosphere at 4°C with anti-TH serum diluted 1:1,000. (Antibody dilutions and washings were carried out in a solution of PBS containing 5% calf serum and 0.1% Triton X-100.) The sections were then washed twice for 10 min, incubated for 2 h at room temperature in sheep anti-rabbit immunoglobulin (Miles Yeda) diluted 1:40, washed again and incubated for 2 h at room temperature in PAP reagent (Dako Immunoglobulins) diluted 1:50. The sections were washed for 10 min as above and then for 10 min in Tris buffer (50 mM, pH 7.1). The peroxidase was reacted with a solution of diaminobenzidine (0.5 mg ml⁻¹, Polysciences) and hydrogen peroxide (0.006%) in Tris buffer (50 mM, pH 7.1), followed by two 10-min washes in distilled water. The slides were mounted in glycerol and viewed with a Zeiss microscope fitted with Nomarski optics. Scale bar, 20 μm .

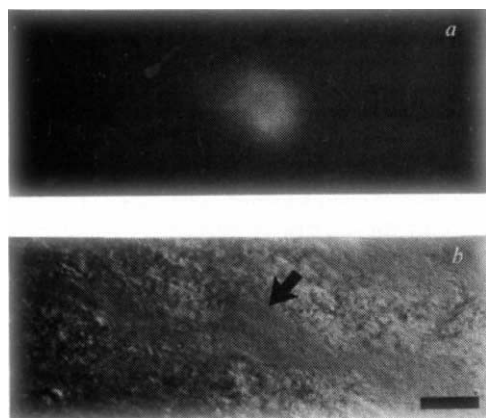


Fig. 2 Glyoxylic acid-induced catecholaminergic fluorescence in an L5 DRG from a 30-day-old rat, viewed with fluorescence (a) and Nomarski optics (b). The animal was injected subcutaneously with colchicine (Sigma, 20 mg per kg) 6 h before the DRG were removed and immediately frozen in OCT, sectioned and processed according to the method of De La Torre¹⁴. The sections were mounted in liquid paraffin and viewed with a Zeiss epi-fluorescence microscope. The arrow shows the fluorescent cell. Scale bar, 20 μm .

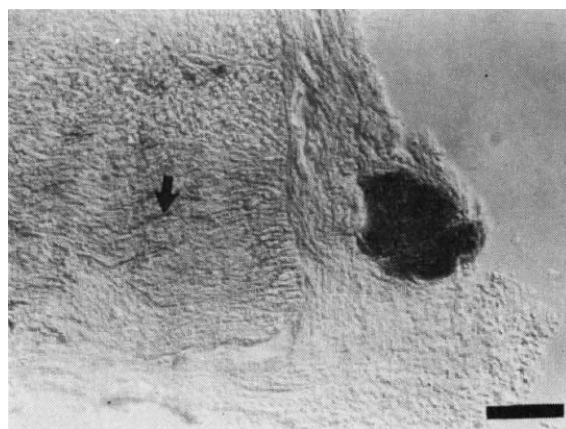


Fig. 3 Light micrograph of a section of DRG of 26-day-old rat showing a group of TH⁺ cells outside the ganglion capsule. The cells lie close to a blood vessel and resemble the paraganglia described in other sensory ganglia¹⁵. Some fibres, in the dorsal root distal to the ganglion, are also stained (arrow); these may be derived from autonomic neurones or from the TH⁺ sensory neurones described in the text. Scale bar, 100 μm .

cells were consistent with their being the same neurones as those containing TH.

In addition to the catecholaminergic neurones within the capsule of the ganglion, we occasionally saw, outside the capsule in the vicinity of blood vessels, small clusters of TH⁺ cells that resembled the paraganglia observed in other sensory ganglia¹⁵ (Fig. 3). Moreover, we observed small numbers of individual TH⁺ cells, presumably neurones, among the fibres of the spinal nerve, distal to the ganglion. Unlike the putative dopaminergic sensory neurones, these extracapsular TH⁺ cells were DBH⁺.

To study the development of the TH⁺DBH⁻ sensory neurones, we examined DRG from rats of various ages between embryonic day 14 and adult using anti-TH immunohistochemistry as described above. No TH⁺ neurones were seen in DRG from animals younger than 9 days post-partum and by 2 weeks of age, the size, number and distribution of TH⁺ cells could not be distinguished from those of the adult. The late appearance of TH in these cells distinguishes them from other catecholaminergic neurones in the central and peripheral nervous systems which produce TH very early in development, typically during embryonic life¹⁶⁻¹⁸.

Although we found no TH⁺ neurones in sections of perinatal rat DRG, cultures of DRG from 16-day embryonic rats (as well as cultures from 8-12-day embryonic chick DRG) did contain small numbers (<5%) of TH⁺ neurones after 24 h *in vitro*. On the other hand, cultures from newborn rats contained no TH⁺ cells. As no neurones were stained with anti-TH in DRG sections at these ages in either species, the source of this early, transient population is unclear. We are now trying to determine whether these cells are a contaminant from some other neural tissue or whether the culture conditions promote this catecholaminergic expression. In either case, it seems unlikely that the TH⁺ cells seen in DRG cultures are related to the putative dopaminergic sensory neurones seen in sections of adult DRG.

As far as we know, this is the first report of catecholaminergic sensory neurones as well as the first demonstration of a qualitative biochemical difference between ganglia at different segmental levels of the spinal cord. The small diameter of the catecholaminergic cell bodies described here suggests that these cells are involved in either pain or temperature pathways but this remains to be determined. The fact that they constitute only a small proportion of the neuronal cell bodies suggests that there may be considerable heterogeneity among the sensory neuronal population with regard to both neurotransmitters and peptide content. Also, the late development of the catecholaminergic phenotype raises the possibility that these

cells have switched the transmitter they make, as it seems unlikely that they could have progressed so far in development without producing any neurotransmitter at all.

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Note added in proof: Katz *et al.*¹⁹ have recently described TH⁺ sensory neurones in rat nodose and petrosal ganglia. We have confirmed this observation and have found the cells in the nodose ganglion to be similar to those reported here in being DBH⁺, but different in already being TH⁺ at birth (unpublished observations).

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Dopamine modulates S-potential amplitude and dye-coupling between external horizontal cells in carp retina

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Horizontal cells in the fish retina are electrically coupled and possess gap junctions^{1,2} so that intracellularly injected dye normally diffuses freely to neighbouring cells^{3–5}. Applied dopamine (DA) alters the spatial properties of the horizontal cell responses to light, increasing the amplitude of photopic L-type S-potentials but decreasing their lateral spread^{6–8}. These effects have been attributed to the action of DA on horizontal cell membrane resistance, particularly at the gap junctions⁷, and our present study on the carp retina agrees with this in showing that DA also restricts intracellular Lucifer yellow (LY) to single injected horizontal cells, an effect, like those of DA on the S-potentials, which is antagonized by the dopamine blocker haloperidol. In addition, we present evidence that dopaminergic interplexiform cells in fish normally function to regulate the spatial properties of responses in horizontal cells, possibly acting on their junctional resistance via a DA-receptor-mediated mechanism. Previous destruction of the interplexiform cells with 6-hydroxydopamine (6-OHDA) resulted in much reduced L-type S-potentials to centred lights but wider lateral spread of these responses, while the dye injected spread extensively to neighbouring cells. After 6-OHDA treatment, however, applied DA retained its normal activity, restoring large-amplitude, narrow receptive-field S-potentials and restricting LY to the injected cells, effects which were both closely mimicked by dibutyryl cyclic AMP.

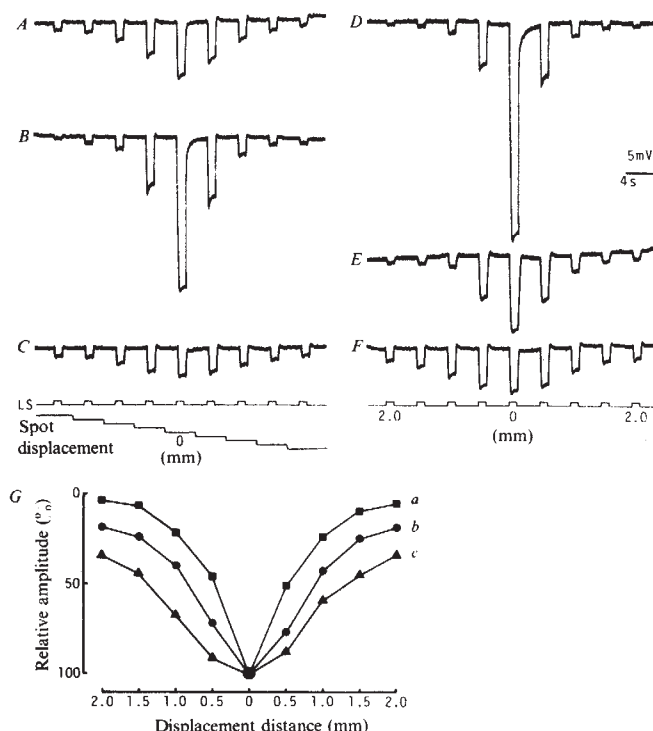


Fig. 1 Changes in S-potential amplitudes with spot displacement. *A*, recorded from a normal retina; *B*, from a retina applied with dopamine (see below); *C*, from a retina from which dopamine (DA) cells had been deprived by intravitreal injection of 6-OHDA 15 days before enucleation (DA cell-deprived retina); *D*, from a DA cell-deprived retina applied with DA; *E*, from a DA cell-deprived retina applied with dibutyryl cyclic AMP; *F*, from a normal retina applied with haloperidol and then DA. In *G*, the normalized mean values of S-potential amplitude are plotted against spot displacement distance (mm). The standard deviation ($n = 5$) is less than 6.4, 18.2 and 14.8% for the points in the normal retina (●, *a*), normal retina applied with DA (■, *b*) and DA cell-deprived retina (▲, *c*), respectively.

Methods: The isolated retina was stimulated by a spot of light beam from a 500-W xenon arc lamp. The stimulus was given with a 1-s duration at 4-s intervals from beneath the preparation. The beam had a set of representative monochromatic interference filters (half-bandwidth 10–15 nm) calibrated so as to deliver, at the retinal surface, 1×10^{13} quanta per cm^2 per s at 621 nm. The interference filters used consisted of 423, 459, 517, 575, 621 and 674 nm. A set of neutral filters was used to attenuate the light intensity in steps of 0.5 log in a range of 4.0–0 log units. A flashing spot (0.5 mm diameter) of red (621 nm; 0.5) light was displaced in steps of 0.5 mm along a straight 4-mm line passing over the recording point (at 0 mm); the bottom tracings indicate the light pulse (LS) and its stepwise displacement. Amplitude (5 mV) and time (4 s) scales are indicated in *D*. To apply chemicals over the entire surface of isolated retinas, they were added to the superfusate^{6,7} or sprayed as a mist jet through a nebulizer over an air-exposed preparation for a brief period¹⁸. Neither method, however, were suitable for the present purpose, because the effect of chemicals applied was transient in general. To determine the most effective condition for this particular purpose, a series of preliminary experiments provided us with a method for applying a certain reagent to the residual vitreous fluid beneath the isolated retinas; we could thus maintain the retinas in a relatively steady condition for 30–90 min under the influence of the reagent applied. The average wet weight of the preparations (retina plus residual vitreous fluid) was 180 mg. Therefore, if the applied DA is assumed to diffuse homogeneously throughout the entire preparation, its concentration is $\sim 10 \mu\text{M}$ in the retina.

To alter S-potential properties, small amounts ($\sim 5 \mu\text{l}$) of Ringer's solution containing DA (2 mM) and pargyline (2 mM) or other DA-related compounds were applied to the residual vitreous fluid beneath the isolated retinas 30 min before electrophysiological experiments. In addition to normal retinas, a special retina preparation was used in which DA cells had been

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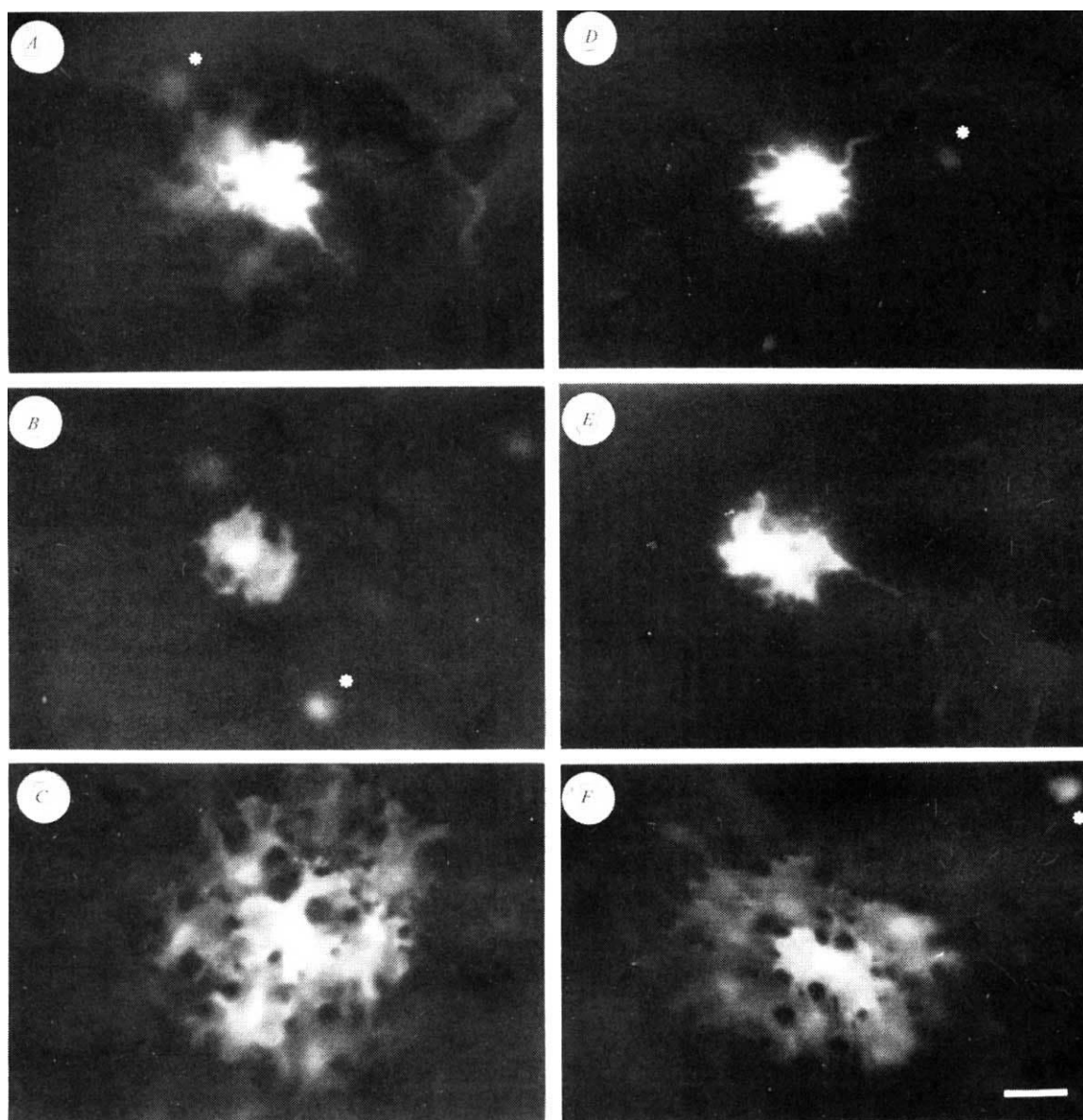


Fig. 2 Fluorescence photomicrographs showing horizontal cells intracellularly marked with Lucifer yellow (LY) and dopamine (DA)-accumulating cells. *A*, a normal retina; *B*, a normal retina applied with DA; *C*, a DA cell-deprived retina; *D*, a DA cell-deprived retina applied with DA; *E*, a DA cell-deprived retina applied with dibutyryl cyclic AMP; *F*, a normal retina applied with haloperidol and subsequent DA.

Methods: LY was iontophoretically injected with a 4 c.p.s. sinusoidal current of ± 10 nA for 15 s into individual horizontal cells, from which photopic L-type S-potentials (ascertained with their spectral response curve¹⁰) were recorded via a glass microelectrode filled with a 4% LY solution³, having a resistance of ~ 200 M Ω after bevelling their tips. As the concentration of LY in solution (4%), the injection time and the electrode resistance were roughly consistent throughout the experiments, the amount of the dye injected was supposed to be the same for each injection. At the end of the electrophysiological experiments, isolated retinas were kept in the dark for 30 min, fixed in a formaldehyde (4%)/glutaraldehyde (0.5%)/sucrose (35%) solution (FGS solution) overnight, and then flatmounted⁹. After examining LY-marked cells in half-dried flatmounts under a fluorescence microscope (Nikon EF), the preparations were softened again in the FGS solution, then subjected to cryosectioning to 15 μ m thickness (not illustrated). The retinal sections were dried over Drierite in a desiccator for several hours, sealed with Entellan and again examined under the fluorescence microscope. In this manner, intracellularly marked horizontal cells as well as DA-accumulating cells, if they exist, were simultaneously seen in both flatmounts and sections of the same preparations. The fluorescence intensity of LY-marked cells varied to some extent depending on the background fluorescence of retinal tissue. One of the endogenously DA-containing cells (marked with *; out of focus) is weakly seen in *A*, while one or some DA-accumulating cells (*) are visible in *B*, *D* and *F*. The cell (*) in *D* belongs to the class of indolamine-accumulating amacrine cells, which also take up applied DA⁹. No fluorescent cells other than those marked with LY are seen in *C* and *E*, because DA was not applied to these DA cell-deprived preparations. For further information, see Fig. 3. Scale bar in *F*, 25 μ m.

destroyed by intravitreal injections of 6-OHDA (20 μ g) plus pargyline (20 μ g) on two successive days 10–20 days before eye enucleation⁹. Isolated normal or DA cell-deprived retinas were placed in a chamber with the photoreceptor side facing up and electrophysiological methods were as in our previous work¹⁰.

The resting potentials of horizontal cells in the dark were approximately -30 to -40 mV in normal retinas and -15 to -30 mV in DA cell-deprived retinas. However, they usually

became hyperpolarized to -60 to -70 mV by DA or related reagents after 1.5–2 h of the beginning of recording, in which case the experiment was terminated. After the experiments, the retinas were processed with a fluorescence histochemical method⁹ to examine horizontal cells intracellularly marked with LY and DA cells in flatmounts and cryosections. 108 Isolated retinas were used; 72 normal and 36 DA cell-deprived retinas. Although three or four cells were marked with LY in each preparation, $\sim 30\%$ of marked points were retrieved in proper

correspondence with the recording sites. Among them, photopic L-type S-potentials were recorded from 104 somata and 44 axon terminals of external horizontal cells.

During an intracellular recording of L-type S-potentials, a spot of monochromatic red (621 nm) light (0.5 mm diameter) was displaced in 0.5-mm steps along a 4-mm line, passing over the recording point at the middle of the displacement length. A representative recording of changes in S-potential amplitude with spot displacement over a normal preparation is illustrated in Fig. 1A. When LY was injected into a horizontal cell in the normal retina, usually five or six cell bodies were seen with an axon terminal (weakly fluorescent in a few flatmounts; Fig. 2A). These cell bodies were located in the external horizontal cell layer when examined in radial cryosections of the retina (not illustrated). Correspondence between electrical responses and marked spots was found in 41 recording sites (cell bodies) explored in 34 normal retinas.

If a small amount ($\sim 5 \mu\text{l}$) of DA (2 mM)-containing Ringer's was applied to the residual vitreous fluid beneath isolated normal retinas 30 min before recording, the amplitude of S-potentials recorded from the illuminated spot (at 0 mm) was very large (Fig. 1B). When the light spot was displaced in steps, the amplitude of S-potentials sharply diminished. In these conditions, the injected LY was found in flatmounts to be restricted within one cell (Fig. 2B), from which the recording was made. This effect was confirmed for each of 13 cell bodies recorded from 10 DA-treated normal retinas. The same procedure was conducted with horizontal cells in DA cell-deprived retinas, from which recorded S-potentials were small in amplitude (Fig. 1C). After intracellular dye injection numerous fluorescent horizontal cell bodies (> 10 cells) were consistently seen (Fig. 2C) and this correlation was established in 18 recording sites (cell bodies) in 12 DA cell-deprived retinas.

When DA was applied to DA cell-deprived retinas ($n = 8$) in the same manner as to normal retinas, high amplitude S-potentials were recorded at 0 mm ($n = 10$ cell bodies), and were sharply reduced in size with spot displacement. Two of the 10 cells exhibited the central response larger than in DA-applied normal retinas (Fig. 1B); one of them is illustrated in Fig. 1D. Correspondingly, the injected LY was found to be restricted within one horizontal cell (Fig. 2D). Similar correlations to these were observed, but less consistently, between S-potentials and dye diffusion areas, when photopic L-type S-potentials were recorded and the dye was found in the axon terminals of horizontal cells¹, in normal and DA cell-deprived retinas.

Dopamine stimulates retinal adenylate cyclase, the enzyme that synthesizes cyclic AMP from ATP^{12,13}. The effect of DA on horizontal cells, observed electrophysiologically in the fish retina, might be mediated by the activation of DA-sensitive adenylate cyclase in the horizontal cell membrane, and if this is the case, dibutyryl cyclic AMP could substitute for DA. A small amount ($\sim 5 \mu\text{l}$) of Ringer's containing dibutyryl cyclic AMP (10 mM) and 3-isobutyl-1-methylxanthine (1 mM) was applied to DA cell-deprived retinas in the same manner as DA. The amplitude of S-potentials at 0 mm was found to be larger (Fig. 1E) than those seen in DA cell-deprived and in normal retinas. The injected LY was found to be restricted within one horizontal cell ($n = 7$ marked sites; Fig. 2E) in four such preparations.

A dopaminergic blocker, haloperidol (10 mM), was similarly applied to eight normal retinas in combination with DA. In this case, haloperidol was given 45 min before DA application, because the former was assumed to affect horizontal cells slowly (30–40 min) although the latter was rapidly effective (a few min). The blocker tended to prevent the appearance of the effect of DA on S-potentials recorded from 15 sites in 6 retinas (Fig. 1F). The reduction in S-potential amplitudes with spot displacement seen in these preparations resembled that shown in Fig. 1C, and the injected LY was found to diffuse through many horizontal cell bodies (Fig. 2F).

The application of DA to normal retinas increased the S-potential amplitude at 0 mm by 2.8 times, from 7.8 ± 0.5 (s.d.)

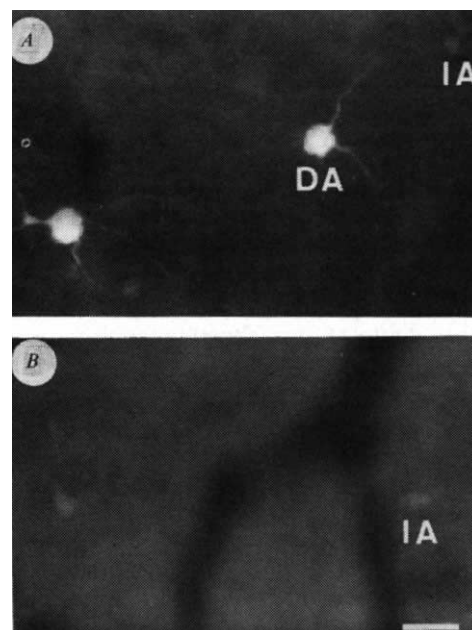


Fig. 3 Fluorescence photomicrographs showing DA-accumulating cells in retinal flatmounts. A, a normal retina applied with DA (DA and smaller-sized IA cells are seen); B, a DA cell-deprived retina applied with DA (only IA cells are seen). Micrographs A and B were obtained from different regions of the same preparations as those shown in Fig. 2B and D, respectively. Scale bar in B, 25 μm .

to 22.0 ± 4.0 mV ($n = 5$). On the other hand, the amplitude of S-potentials recordable from DA cell-deprived retinas was 4.7 ± 0.7 mV, which was 60% of the normal amplitude. In Fig. 1G, the normalized mean values of S-potential amplitude, obtained from five representative records in each preparation, are plotted as a function of displacement distance (mm) of the light spot. This indicates that the receptive field of S-potentials seems to be reduced by DA, the lack causing the opposite. The curves of normalized amplitude of S-potentials, shown in Fig. 1E (DA cell-deprived retina applied with dibutyryl cyclic AMP) would fall between the curves obtained from normal and DA-applied normal retinas, while that in Fig. 1E (haloperidol-applied normal retina) would be interplacated between the curves of normal and DA cell-deprived retinas in Fig. 1G.

As the external horizontal cells marked with LY- and DA-accumulating cells are located in different retinal layers, the latter can be detectable out of focus in Fig. 2 (labelled with stars). Figure 3 shows, for example, DA-accumulating cells, A being taken from the same preparation as in Fig. 2B (normal retina applied with DA) and 3B from the same as in Fig. 2D (DA cell-deprived retina applied with DA).

The neurotoxin 6-OHDA is assumed selectively to destroy catecholamine neurones and their nerve terminals in the central nervous system^{14,15}. In the fish, indolamine-accumulating (IA) cells are found to remain intact after 6-OHDA treatment (Fig. 3B)^{9,16}, and in the present experiments, S-potentials in the 6-OHDA-treated retinas were restored by applied DA to the amplitudes normally caused by this agent, indicating that the postsynaptic site of the horizontal cell membrane remains intact after neurotoxic destruction of DA cells.

All the apparent effects of DA observed in the present study (the increased amplitude of the central receptive field response, reduced receptive field and abolition of dye diffusion) can be explained on the basis of an uncoupling action of DA at gap junctions between horizontal cells. Recently, DA has been reported markedly to reduce the receptive field of horizontal cells and at the same time to increase both their membrane and coupling resistances in the teleost retina¹⁷. The absence of DA from the retina may reduce the membrane and coupling resistances, resulting in the reduced amplitude of S-potentials, enlarged receptive field and wider dye-coupling area.

A clearcut correlation between electrical and dye coupling has been demonstrated in external horizontal cells of the turtle retina⁵, where γ -aminobutyric acid (GABA) is assumed to modulate the spatial properties of S-potentials. The DA cells are amacrine type in the turtle retina, differing from the interplexiform cells in the carp retina. However, DA in the carp and GABA in the turtle seem to have a similar role in modulating S-potentials of external horizontal cells. Finally, another difference between findings in fish and results from the turtle retina is of interest⁵. Dye injected into a horizontal cell body mainly diffuses to neighbouring cell bodies in the carp retina, whereas it seems to spread from a cell body to the axon terminal first and then diffuses to neighbouring terminals in the turtle. Therefore, gap junctions at the horizontal cell body level may predominate in electrical coupling in the carp while junctions between axon terminals have a similar role in the turtle.

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Coffee contains potent opiate receptor binding activity

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Opiate receptor-active peptide fragments (exorphins) have been identified recently in casein¹ and gluten² hydrolysates, and morphine has been found in bovine and human milk³. To determine whether similar peptides or alkaloids occur in other foodstuffs, we have screened potential sources using a rat brain homogenate assay to detect opiate receptor activity. We report here that instant coffee powders from a variety of manufacturers compete with tritiated naloxone for binding to opiate receptors in the rat brain membrane preparations, with no significant difference between normal and decaffeinated coffee. The receptor binding activity resembles that seen with opiate antagonists, in that there was no change in the half-maximal effective dose (ED_{50}) in the presence of 100 mM Na^+ ; on bioassay, the activity was similarly shown to be antagonistic and specific for opiate-induced inhibition of twitch. Preliminary characterization of the activity reveals that it has a molecular weight (MW) in the range 1,000-3,500, is heat-stable, ether-extractable, not modified by enzymatic digestion with papain, and clearly separable from caffeine and morphine on TLC. As its concentration in an average cup of coffee is five times the ED_{50} , these data suggest that drinking coffee may be followed by effects mediated via opiate receptors, as well as effects of caffeine.

Table 1 Inhibition of 3H -naloxone binding by the products of preliminary characterization experiments

Experiment/(fraction)	% Inhibition
Dialysis	
(Dialysate)	71
(Retentate)	-1
Extraction	
(Ether)	80
(<i>n</i> -Butanol)	11
(Methanol)	51
Preparative TLC of ether extract	
(R_F 0.20-0.30)	21
(R_F 0.30-0.35)	63
(R_F 0.35-0.48)	69
(R_F 0.48-0.58)	24
Heat treatment	92
Enzymatic digestion	76

Total binding represents 11.3-12.9% of counts added; nonspecific binding in the presence of 1,000-fold excess of naloxone represents 0.9-1.4% counts added, or 8-11% of total binding. For molecular weight studies, aliquots (8 ml) of 100 mg ml⁻¹ aqueous solution of coffee powder were placed in presoaked Spectrapor 3 dialysis tubing (cut-off MW 3,500), dialysed overnight against 160 ml of 0.05 M Tris-HCl, 0.5% bovine serum albumin (BSA), pH 7.4, the buffer changed three times and the dialysates pooled, lyophilized and redissolved in 8 ml distilled water. In a second study, 200 ml of coffee powder solution, clarified by two passes through Millipore prefilters, were filtered through a presoaked Amicon UM-2 ultrafiltration membrane (cut-off MW 1,000). Solvent extractions with ether, water-saturated *n*-butanol and methanol were performed using Merck Extralut kieselguhr columns; 20 ml of an aqueous solution of instant coffee were applied to the column and allowed to equilibrate for 15 min. Diethyl ether (80 ml) was then run through the column, collected and evaporated to dryness under reduced pressure. This wash was followed by 60 ml *n*-butanol; final recovery of column adsorbents was achieved by washing the column packing in a large Buchner funnel with 500 ml methanol. Preparative TLC of ether-extractable material was carried out on silica gel and developed in chloroform/ethanol (4:1). Caffeine, theophylline, theobromine and morphine base were run as standards. For stability studies, instant coffee solution was boiled for 10 min, or 500 mg of papain in 5 ml Tris-HCl pH 7.4 were added to 500 mg of instant coffee powder, and incubated at 60 °C for 1 h. The enzyme was retained by ultrafiltration through Millipore PTGC membranes (cut-off MW 10,000), and the filtrate assayed.

Table 2 Competition for 3H -naloxone binding sites

Competitor	ED_{50} ($-Na^+$)	ED_{50} (Na^+ , 100 mM)	Ratio ($+Na^+/-Na^+$)
Naloxone	3.4 nM	3.2 nM	0.9
(antagonist)			
Morphine	10.8 nM	60.5 nM	5.6
(agonist)			
Normal instant coffee	1.2 mg ml ⁻¹	1.2 mg ml ⁻¹	1.0
Decaffeinated instant coffee	1.6 mg ml ⁻¹	1.1 mg ml ⁻¹	0.7

ED_{50} values are shown for naloxone, morphine sulphate, and normal and decaffeinated instant coffee in the absence and presence of 100 mM Na^+ .

Solutions of instant coffee powders, both normal and decaffeinated, showed dose-related displacement of 3H -naloxone binding over a range of concentrations up to 10 mg ml⁻¹. An extract, partially purified on Sep-pak C₁₈ cartridges (see below), showed parallel, though more potent, dose-related displacement (Fig. 1). Similar studies using 3H -D-Ala-L-Met-enkephalinamide or 3H -D-Ala-D-Leu-enkephalin as tracer gave identical results to those obtained in the 3H -naloxone displacement assay; four proprietary brands of normal instant coffee, two of decaffeinated coffee, and coffee brewed from freshly roasted beans showed similar activity. Instant tea, cocoa, soup powders and stock cubes, and extracts of yoghurt and cream cheese, were without activity in the assay. Caffeine,

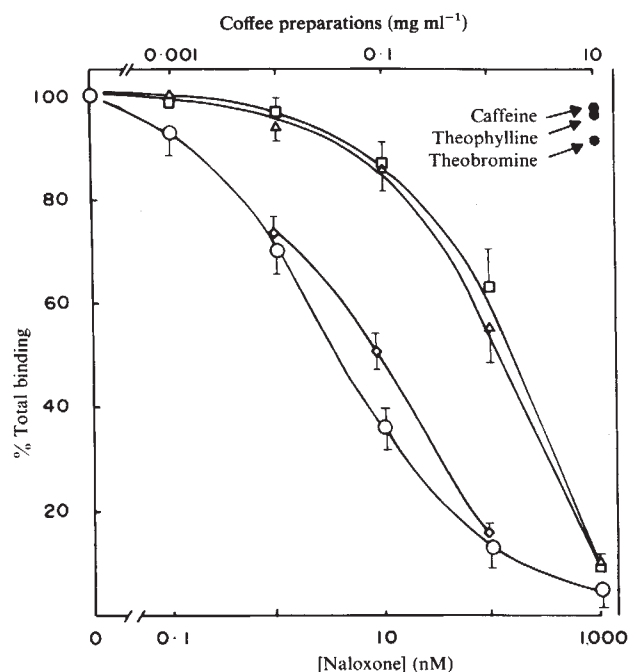


Fig. 1 Displacement curve for binding of ^3H -naloxone to rat brain opiate receptors. The curves are the mean $\pm$ s.e.m. (vertical bars), $n = 10$ ($\circ$, unlabelled naloxone) or $n = 3$ (Δ ; whole coffee; $\square$, decaffeinated coffee; and $\diamond$, Sep-pak extract). Solutions of whole and decaffeinated coffee powders were prepared by dilution of stock solutions which had been clarified by filtration through Millipore prefilters. The Sep-pak extract used was prepared by fractionation of whole coffee solutions on C_{18} Sep-paks; 2 ml of a 100 mg ml^{-1} solution were applied to the cartridge and eluted by 5-ml volumes of a stepwise gradient from 0% to 100% methanol in 20% steps. The eluates were dried under reduced pressure and made up in 400 μl vols of 0.05 M Tris-HCl pH 7.4 buffer. Triplicate determinations of activity in the opiate receptor assay were carried out at several dilutions. The receptor assay system was based on that of Pert and Snyder⁴. Male Sprague-Dawley rats were killed by decapitation, and brains minus cerebellum homogenized in $25\times$ vol of 0.05 M Tris-HCl, pH 7.4 buffer using a Brinkmann Polytron on setting 4 for 5 s. The homogenate was centrifuged for 20 min at 18,000g, and the pellet resuspended in $25\times$ vol buffer. Aliquots (0.5 ml) of this preparation were incubated with 1 nM ^3H -naloxone (40 Ci mmol^{-1} ; NEN) alone, with various concentrations of nonradioactive opiate competitors, or with preparations of instant coffee powders or extracts. Incubations were done in 0.5% BSA and 10% Trasylol for 30 min at 25°C , and terminated by rapid vacuum filtration through Whatman GF/B glass fibre filters pre-wetted with buffer containing 0.5% BSA. Filters were washed with 5 ml buffer, air-dried and added to 10 ml non-aqueous scintillant. Total binding was $13.53 \pm 0.84\%$ of counts added (mean $\pm$ s.e.m., $n = 10$). All results shown are corrected for adsorption of radioactivity to filters ($1.42 \pm 0.29\%$ of counts added, mean $\pm$ s.e.m., $n = 10$).

theophylline and theobromine, up to 1 mM, did not displace ^3H -naloxone. The equivalent potency of normal and decaffeinated coffee is an additional argument against caffeine as the source of the activity, and is in agreement with the work of Pert and Snyder⁴ who found no reduction of ^3H -naloxone binding by caffeine at 0.1 nM.

Table 1 summarizes the results of preliminary characterization experiments. The opiate receptor activity in coffee powders traversed dialysis tubing (cut-off MW 3,500), but was retained by ultrafiltration membranes (cut-off MW 1,000). The activity was stable to both heat and treatment with pepsin, a proteolytic enzyme with broad specificity. In organic solvent extractions on Extralut columns, the bulk of the activity was eluted with a diethyl ether wash. Part of the activity remained adsorbed to the column matrix, despite butanol washing, and appeared only on further washing with large volumes of methanol. Extractions of the activity were also performed on

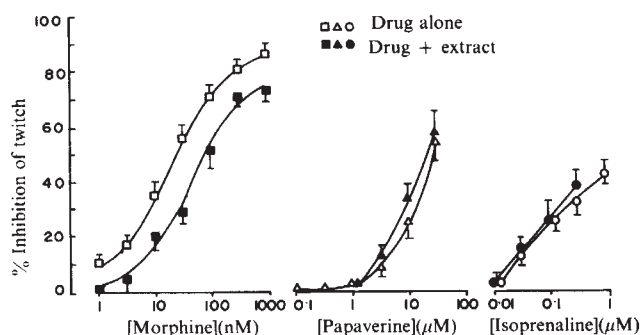


Fig. 2 Effect of addition of $20 \mu\text{g ml}^{-1}$ Sep-pak extract (40% methanol) on morphine-, papaverine- and isoprenaline-induced inhibition of the electrically induced twitch in the myenteric plexus-longitudinal muscle strip from guinea pig ileum (0.5 ms, 0.1 Hz, 60 V; Krebs-Ringer, 37°C) described in detail elsewhere⁸. The ordinate represents the percentage inhibition of control twitch, and the abscissa the concentration of drug: $\square$, morphine alone; $\blacksquare$, morphine plus extract; $\triangle$, papaverine alone; $\blacktriangle$, papaverine plus extract; $\circ$, isoprenaline alone; $\bullet$, isoprenaline plus extract. Values shown are mean $\pm$ s.e.m., $n = 4-6$.

small reverse-phase C_{18} cartridges (Sep-paks; Waters Associates) using a stepwise gradient of methanol/water from 0 to 100% methanol. The activity was eluted from the cartridges by 40–60% methanol with slightly more material present in the 40% fraction.

The ether-extractable material was characterized further by preparative TLC on silica gel. The activity migrated as a broad band (R_F 0.30–0.48) clearly separable from caffeine (R_F 0.50–0.55) and morphine (R_F 0.1). Material in the ether extract migrating at R_F 0.50–0.55 (assumed to include caffeine) had no opiate receptor activity.

On the criteria of Pert *et al.*⁵ for discrimination between opiate agonist-antagonist behaviour, both regular and decaffeinated instant coffee powders showed antagonist binding behaviour, with no significant shift in ED_{50} in the presence of 100 mM Na^+ . Morphine, a pure agonist, showed a sixfold shift in parallel studies (Table 2). In agreement with these binding data, the Sep-pak extract (40% methanol) displaced to the right a morphine dose-response curve in guinea pig ileum. This effect was opiate receptor-specific in that equivalent concentrations of coffee extract had no effect on the inhibition of electrically induced contraction caused by papaverine or isoprenaline (Fig. 2). The coffee extract alone did not affect contractions of the guinea pig ileum.

These data suggest the presence of one or several components in instant coffee powders that compete with ^3H -naloxone for rat brain opiate receptors. The activity is of low molecular weight (1,000–3,500), ether-extractable and stable to heat and enzymatic digestion. It exhibits antagonist behaviour and is neither morphine nor caffeine. The stability to heat and enzymatic treatment suggests that the active factor is unlikely to be a peptide(s), as is the case for opiate receptor-active fragments in gluten and casein hydrolysates^{1,2}. The antagonist characteristics in both the binding and bioassay systems and the clear chromatographic separation from morphine base on TLC, make it unlikely that the predominant opiate receptor activity in coffee powders is morphine.

Further purification studies are required to determine whether the activity represents low concentrations of a high-affinity opiate receptor ligand or higher concentrations of a low-affinity ligand. Whatever the affinity and abundance of the ligand, the concentration for half-maximal naloxone displacement (1.3–1.7 mg powder per ml) is one-fifth the level in a cup of coffee prepared according to the manufacturer's recommendations.

If the volume of distribution of an intravenous dose of naloxone is taken as 12 l, a cup of coffee contains the equivalent of one-third of an ampoule of naloxone. Caffeine has no effect

in the opiate receptor assay, at concentrations equal to those found in the instant coffee powder preparations used. It has, however, recently been suggested that caffeine stimulates the release of immunoreactive β -endorphin via opiate receptor mechanisms, in that this stimulation is naloxone-inhibitable⁶; these authors suggested that the popularity of caffeine as a component of many drinks may be related to this β -endorphin-releasing activity. The finding (ref. 4 and Fig. 1) of minimal binding of caffeine to opiate receptors suggests that the effect of caffeine on β -endorphin release may be mediated by a series of steps, one involving an action of caffeine via non-opiate mechanisms⁷, and another including a naloxone-inhibitable opiate receptor interaction. Further, our finding suggests that coffee, in addition to possibly releasing endogenous β -endorphin, contains significant levels of at least partially antagonist opiate receptor activity. Further experiments are needed to determine how this activity might affect the coffee drinker. The activity may be primarily on gastrointestinal opiate receptors; alternatively, the exorphin activity in instant coffee may cross from the gut into the circulation, and despite the dilution involved, interact with more distal opiate receptors, including those in the central and peripheral nervous systems.

The chemical identity of the putative antagonist exorphin in coffee remains to be established; from the binding and bioassay studies described here, however, it seems that the activity is both coffee-specific and opiate receptor-mediated.

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Caffeine induces a transient inward current in cultured cardiac cells

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Electrical excitation of cardiac muscle may sometimes be due to initiation of inward current by the presence of Ca^{2+} ions at the inner surface of the cell membrane. During digitalis toxicity and other conditions that abnormally augment cellular Ca^{2+} stores, premature release of Ca^{2+} from the sarcoplasmic reticulum leads to a transient inward current, which is large enough to initiate premature beats and is accompanied by a transient contractile response^{1–5}. This inward current may be mediated either by electrogenic sodium-calcium exchange⁶ or by specific Ca^{2+} -activated cation channels that have recently been characterized in tissue cultures of cardiac myocytes⁷. An obvious question raised by these observations is whether release of the sequestered Ca^{2+} stores during each normal beat exerts a similar influence on membrane potential. To explore this, chick embryonic myocardial cell aggregates were voltage-clamped during abrupt exposure to caffeine, which is known to release Ca^{2+} from the sarcoplasmic reticulum^{8–10}. The speed of the perfusion system and the relative absence of diffusion barriers in the tissue-cultured cells allowed the effects of caffeine-induced Ca^{2+} release to be studied on a time scale comparable to that of a single normal beat. We report here that abrupt exposure of the cells to caffeine produced a transient inward current having similar features to that of digitalis toxicity, and which was both large enough and rapid enough to potentially contribute to the action potential.

Chick embryonic ventricular cells were obtained by trypsinization of 9–12-day ventricular fragments as described previously^{11,12}. Cells cultured on a hydrophobic surface formed spontaneously beating spheroidal aggregates 60–150 μm in diameter. After 3–10 days in culture, these aggregates were plated on glass slides, immersed in physiological saline on the stage of an inverted microscope and penetrated with microelectrodes. Intracellular recordings were obtained either in current or voltage clamp mode using a Dagan 8100 single electrode voltage clamp at a sampling frequency of 500 Hz. Membrane potential during voltage clamp was always held in the physiological pacemaker range, so that the largest applied currents were <15 nA. Adequacy of membrane potential control was verified in several experiments by an independent intracellular electrode (see Fig. 1A). Aggregates were superfused using a Buchler peristaltic pump with effluent catheters of inner diameter 280 μm , and were positioned 100 μm from the aggregates. Activation of the pump at a flow rate of 1.6 cm s^{-1} (60 $\mu\text{l min}^{-1}$) caused the aggregate to be engulfed by the superfusate stream in <1 s (see ref. 13). Mechanical activity was sometimes recorded by a photodiode placed in the image plane of an eyepiece lens, whose output was proportional to the instantaneous displacement of a 30- μm segment of the aggregate's edge¹². Data were recorded with an oscilloscope camera, or with a Gould-Brush 2400 strip-chart recorder. Current was normalized to membrane area by the method of Nathan and DeHaan¹⁴.

In Fig. 1B, a transient inward current was initiated by abrupt superfusion of a voltage-clamped myocardial cell aggregate with 10 mM caffeine (arrow b). Caffeine-induced inward current was elicited several times in the same aggregate, and ranged in amplitude from 0.6 to 4.7 $\mu\text{A cm}^{-2}$ in 10 aggregates clamped at -70.3 ± 2.2 mV. The caffeine-induced inward current rose from baseline to peak in 121 ± 13 ms, and decayed in ~ 4 s. Decay of the inward current was roughly exponential, but several smaller inward current peaks were often superimposed on the falling phase (Figs 1C, 2B, 3A). Like the digitalis-induced transient inward current³, the caffeine-induced current was reduced or abolished when extracellular sodium was replaced by Tris. In Fig. 2A, abrupt superfusion with Na^+ -free, Tris saline caused a temporary outward shift in membrane current. Addition of 10 mM caffeine after 35 s in low Na^+ caused a barely discernible inward current that was about sixfold smaller than control currents elicited in the same aggregate before Na^+ removal (Fig. 2B, left), and after Na^+ restitution (Fig. 2B, right). Similar results were obtained in four other aggregates.

The caffeine-induced inward current could be ascribed to intracellular Ca^{2+} release because it was accompanied by an obvious mechanical response. This mechanical response was not a smooth, synchronous contraction like that observed during normal beating¹², but consisted of asynchronous 'miniature twitches', each involving a small portion of the aggregate. These twitches were most frequent at the onset of caffeine exposure and subsided within a few seconds, together with the inward current. To characterize the mechanical response further, optical recordings were obtained from small regions of voltage-clamped aggregates during caffeine exposure. Figure 3A (lower trace) shows that caffeine exposure induced a unitary twitch, the duration of which (350 ms) was comparable both to the physiological contractions and to the subsidiary peaks often seen in the caffeine-induced inward current. These observations suggest that the caffeine response does not result from uniform changes in intracellular Ca^{2+} throughout the aggregate, but reflects the summated effects of localized, asynchronous Ca^{2+} release. Spontaneous electrical and mechanical oscillations in cardiac preparations intoxicated with digitalis¹⁵ or veratrine¹⁶ are thought to have a similar basis.

The lack of synchrony of the caffeine response may be due to delayed diffusion of caffeine into the interior of the aggregate. Such delays would also lead to underestimation of the current inducible by caffeine, as only the superficial portion of the aggregate could contribute to the initial current peak. If each

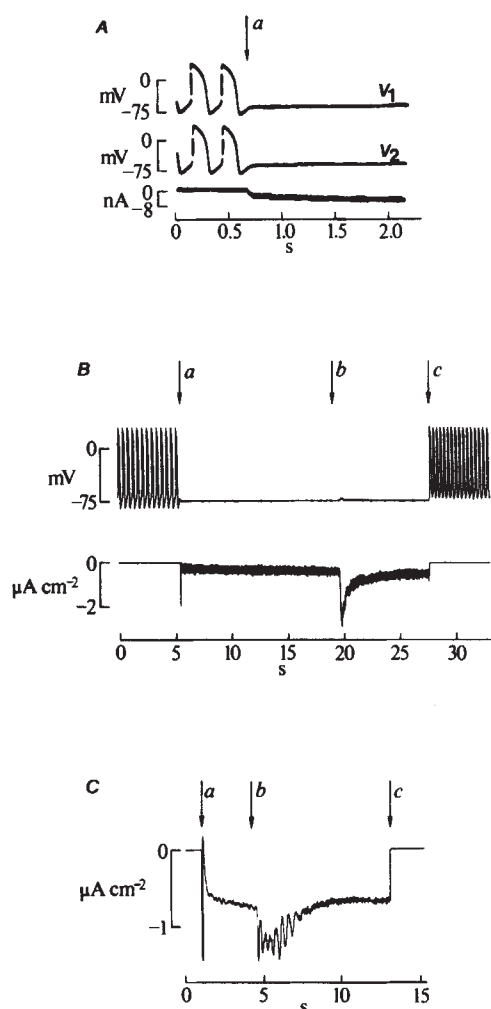


Fig. 1 Ionic currents elicited in chick embryonic myocardial cell aggregates by abrupt superfusion with caffeine. **A** illustrates the homogeneity of membrane potential control achieved by the single electrode voltage clamp in a 110- μm diameter aggregate. V_1 was recorded by the current-passing electrode, while V_2 was recorded by an independent electrode inserted on the opposite side of the aggregate. Activation of the voltage clamp circuitry at arrow *a* elicited the expected time-dependent pacemaker current (bottom trace). Membrane potential was similarly well controlled during abrupt voltage steps, provided that the fast Na^+ current was not activated. In **B**, an aggregate was voltage-clamped at -74 mV between arrows *a* and *c*, and abruptly superfused with 10 mM caffeine, beginning at arrow *b*. Caffeine exposure induced an abrupt transient inward current. **C** is from a similar experiment in which subsidiary inward current peaks were especially prominent. All recordings were obtained in physiological saline consisting of 137 mM NaCl, 2.7 mM KCl, 0.4 mM NaH_2PO_4 , 1.8 mM CaCl_2 , 1.0 mM MgCl_2 , 5.5 mM dextrose, 6.0 mM HEPES pH 7.4, at $37 \pm 0.2^\circ\text{C}$.

aggregate were a sphere of radius r , and if, at the time of the initial current peak, a sufficient concentration of caffeine to trigger Ca^{2+} release had penetrated a uniform distance, x , into the aggregate's interior, then the proportion of the cell mass potentially contributing to the peak current would be given by:

$$\frac{4/3\pi r^3 - 4/3\pi(r-x)^3}{4/3\pi r^3} = 3\left(\frac{x}{r}\right) - 3\left(\frac{x}{r}\right)^2 + \left(\frac{x}{r}\right)^3 \quad (1)$$

The value of this expression varies inversely with r for values of $r > x$. Figure 4 shows the peak amplitude of the caffeine-induced current, relative to membrane area, plotted against the reciprocal of aggregate radius (r^{-1}) for 10 aggregates. The inverse relation between aggregate radius and normalized peak

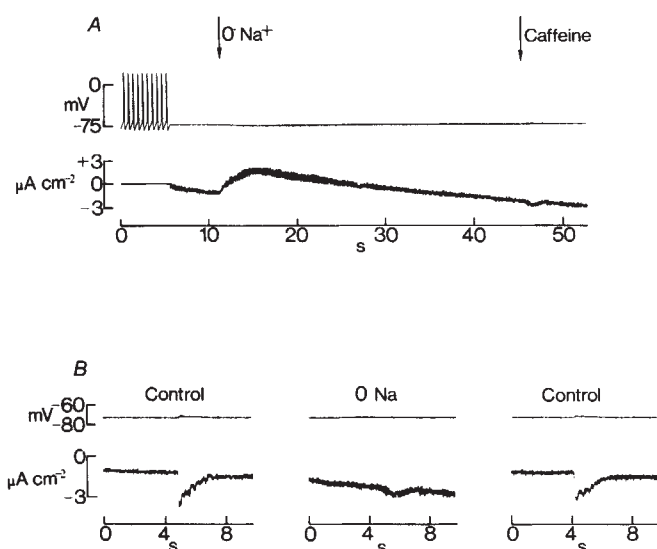


Fig. 2 Dependence of the caffeine-induced inward current on extracellular Na^+ . In **A**, an aggregate was superfused with Na^+ -free, Tris saline beginning 6 s after activation of the voltage clamp. Na^+ removal produced an early outward shift in membrane current, which gradually decayed; 35 s after Na^+ removal, the superfusate was switched to a Na^+ -free solution containing 10 mM caffeine (second arrow). Only a minimal inward current was elicited. **B** shows the inward current elicited in the same aggregate by exposure to 10 mM caffeine in the presence of normal extracellular Na^+ . Recordings obtained several minutes before Na^+ removal (left-hand record), and several minutes after Na^+ restitution (right-hand record) are compared with the response in the absence of Na^+ (middle record), which is enlarged from **A**.

current is in general agreement with the predicted effect of diffusion delays on response amplitude. Despite the loss of synchrony engendered by diffusion delays, the current inducible by caffeine was, nevertheless, large enough to affect membrane potential appreciably during normal spontaneous beating. In Fig. 3C, hyperpolarizing current pulses equal in amplitude to the caffeine-induced current of Fig. 3A were applied to the same aggregate several minutes after cessation of caffeine exposure. The current pulses increased the maximum diastolic potential by 17 mV, and reduced the duration of the action potential at threshold by 33%.

The experiments reported here show that abrupt exposure of myocardial cell aggregates to caffeine induces a significant inward current, which is similar to the transient inward current of digitalis toxicity in three respects: (1) Both currents are accompanied by a contractile response which cannot be suppressed by clamping the membrane in the diastolic potential range¹. (2) Both currents seem to be driven by abrupt Ca^{2+} release events, which can occur independently in small regions of the preparation, giving rise to asynchronous mechanical activity, and accompanying irregularities in the total membrane current¹⁵. (3) Both currents are reduced by removal of extracellular Na^+ ions.

The suppression of the caffeine-induced inward current in low extracellular Na^+ does not necessarily establish Na^+ as the charge carrier. It is possible, for example, that Na^+ removal causes an increase in intracellular free Ca^{2+} , thereby occluding the Ca^{2+} -activated conductance. Alternatively, Na^+ removal might cause depletion of intracellular Ca^{2+} if resting Ca^{2+} influx were normally coupled to Na^+ efflux. These phenomena could also contribute to the Na^+ -sensitivity of the transient inward current of digitalis toxicity, although they would not account for the Na^+ -sensitivity of the reversal potential³.

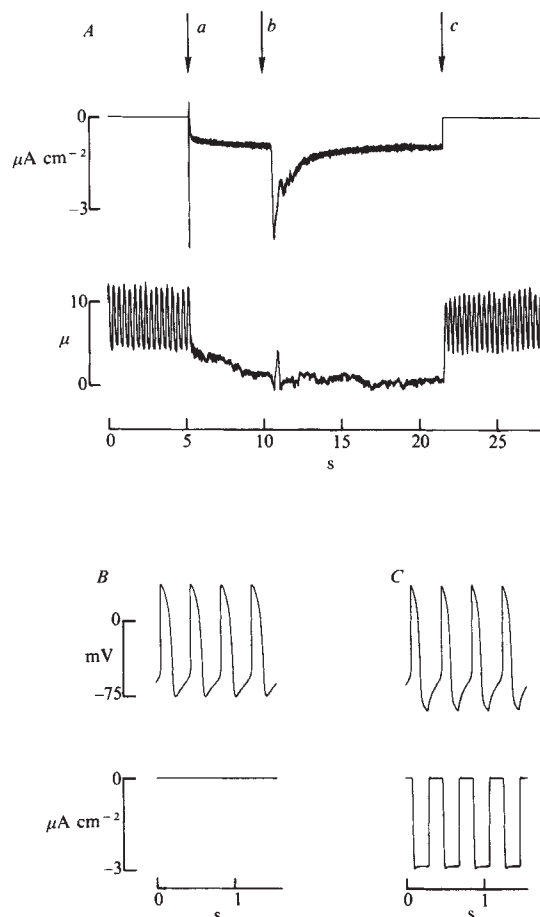


Fig. 3 *A*, effects of abrupt caffeine exposure on mechanical activity in a small region of a myocardial cell aggregate. The upper trace is membrane current, the lower trace edge displacement (in μm), recorded by the photodiode. The aggregate beats spontaneously, except between arrows *a* and *c*, when membrane potential was clamped at -69 mV . Cessation of beating was followed by a slow component of mechanical relaxation, as described previously^{11,12}. Abrupt superfusion with 10 mM caffeine, beginning at arrow *b*, produced a brief twitch. Direct observation of aggregates during similar experiments revealed that caffeine-induced twitches occurred asynchronously in discrete regions of the aggregate during the first few seconds of exposure. *B* and *C* show that the current inducible by caffeine in *A* was large enough to affect membrane potential significantly during normal beating. The action potentials in *B* were recorded several minutes after cessation of caffeine exposure. In *C*, hyperpolarizing current pulses equal in amplitude to the caffeine-induced inward current in *A* were applied at the same frequency as spontaneous beating. Each current pulse began just after the upstroke of an action potential, and reduced action potential duration at threshold by 33%. Depolarizing current pulses of appropriate amplitude produced comparable prolongation of the action potential in several experiments.

The discreteness of the electrical and mechanical oscillations that occur in digitalis toxicity has been ascribed to regenerative, Ca^{2+} -induced Ca^{2+} release, which can occur repetitively in skinned cardiac fibres exposed to slightly elevated free Ca^{2+} concentrations¹⁷. Caffeine may trigger the same mechanism on a once only basis. This interpretation is supported by the fact that 10 mM caffeine abolishes the transient inward current in Purkinje fibres in which Ca^{2+} overload has been induced by removal of external potassium¹⁸.

An important implication of the present study is that Ca^{2+} release also produces significant ionic permeability changes in

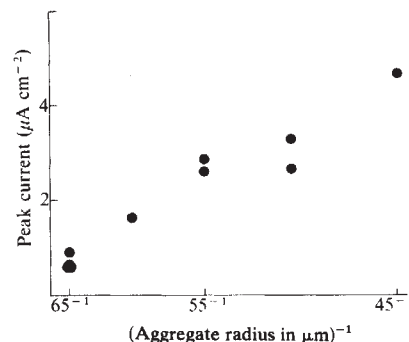


Fig. 4 Inverse relationship between the relative amplitude of the caffeine-induced current and the radius of 10 cell aggregates clamped at -70.3 ± 2.2 ($\pm\text{s.e.}$) mV . The caffeine-induced current (ordinate) is expressed as peak current minus holding current, divided by total cell membrane area. Membrane area is estimated as $(4/3)k\pi ab^2$, where *a* and *b* are the major and minor semi-axes of the aggregate (in μm) and *k* is $4.4 \times 10^{-9}\text{ cm}^2\mu\text{m}^{-3}$, a constant derived from the histological data of Nathan and DeHaan¹⁴. The reciprocal of each aggregate radius is indicated on the abscissa, the largest semi-axis being used for four aggregates that did not appear spherical. Caffeine should induce relatively little current in large preparations because only the superficial cells could contribute to the initial current peak.

conditions in which the sequestered stores have not been artificially augmented. The action potential plateau, for example, is produced by a 'slow inward current' that does not completely inactivate¹⁹, and is thought to be carried by both Na^+ and Ca^{2+} ions. It has been proposed that the component of the slow inward current carried by Na^+ ions could be mediated by a separate, Ca^{2+} -activated permeability mechanism²⁰. The present results support this hypothesis by showing that release of normally sequestered Ca^{2+} produces a Na^+ -sensitive inward current that is large enough to affect membrane potential significantly and has a relatively rapid onset. Although the largest caffeine-induced current in Fig. 4 is about fivefold smaller than the maximum slow inward current in myocardial cell aggregates¹⁴, depolarization could induce a larger Ca^{2+} -activated conductance than caffeine, due to greater synchrony of Ca^{2+} release and depolarization-induced Ca^{2+} influx^{6,19}.

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Primary structures of β - and δ -subunit precursors of *Torpedo californica* acetylcholine receptor deduced from cDNA sequences

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The nicotinic acetylcholine receptor (AChR) from fish electric organ and mammalian skeletal muscle is the best characterized neurotransmitter receptor (reviewed in refs 1–3). The AChR from the electroplax of the ray *Torpedo californica* consists of five subunits present in a molar stoichiometry of $\alpha_2\beta\gamma\delta$ (refs 4–6); the apparent molecular weights of the α -, β -, γ - and δ -subunits are 40,000 (40K), 50K, 60K and 65K, respectively^{7–11}. Knowledge of the primary structures of these constituent polypeptides would facilitate the understanding of the molecular mechanism underlying the function of the neurotransmitter receptor. Recently, we have cloned cDNA for the α -subunit precursor of the *T. californica* AChR and have deduced the primary structure of this polypeptide from the nucleotide sequence of the cloned cDNA¹². Here we report the cloning and nucleotide analysis of cDNAs for the AChR β - and δ -subunit precursors. The primary structures of the two polypeptides deduced from the cDNA sequences reveal conspicuous amino acid sequence homology among these and the α -subunits. The three subunits contain several highly conserved regions which may be essential for the receptor function or inter-subunit interaction.

The procedure used to clone cDNAs for the AChR β - and δ -subunit precursors was similar to that used to clone the α -subunit precursor cDNA¹² (see Fig. 1 legend for experimental details). A library of cDNA clones derived from poly(A) RNA from an electric organ of *T. californica* was constructed using the plasmid DNA vector of Okayama and Berg¹³. To screen the library for cDNAs encoding the β - and δ -subunit precursors, we used two kinds each of synthetic oligodeoxyribonucleotide mixtures which represent all possible cDNA sequences predicted from known amino acid sequences in the amino-terminal region of these subunits⁶. cDNA clones that hybridized with both probes were isolated for each subunit precursor. The nucleotide sequences of the cloned cDNAs were determined by the method of Maxam and Gilbert¹⁴ according to the strategy indicated in Fig. 1.

Figure 2a, b shows the primary structures of the *T. californica* mRNAs encoding the β - and δ -subunit precursors of the AChR, respectively, deduced from the cDNA sequences. For both subunit precursors, the nucleotide sequence of the whole protein-coding region was determined with at least two clones. The amino-terminal 54 amino acids of the β -subunit and the amino-terminal 56 amino acids of the δ -subunit, determined by protein sequencing⁶, are encoded by nucleotide residues 1–162 and 1–168 of the corresponding mRNAs, respectively, except that a threonine residue, instead of the arginine residue at position 51 of the β -subunit⁶, is predicted by the mRNA sequence. The amino acid sequences of the cryptic portions of the β - and δ -subunit precursors were deduced from the mRNA

sequences by using the reading frame corresponding to the known amino acid sequences.

The translation initiation site of the δ -subunit precursor was assigned to the methionine codon AUG, represented by nucleotide residues –63 to –61, because this is the first AUG triplet that appears downstream from the nonsense codon UGA (residues –162 to –160) found in frame. The translation initiation site of the β -subunit precursor was tentatively assigned to the methionine codon at residues –72 to –70, which is the first AUG triplet within the mRNA sequence deduced, because the sequence of the 24 amino acid residues starting with this methionine exhibits a feature common to the prepeptides of the other AChR subunits (see below). The codon specifying the alanine residue at position 469 of the β -subunit precursor is followed by the translation termination codon UGA, and the codon specifying the alanine residue at position 501 of the δ -subunit precursor by the termination codon UAA. The 3' untranslated region of the β -subunit precursor mRNA (200 nucleotides long) contains the polyadenylation signal AUUAAA (residues 1,587–1,592) 16 nucleotides upstream from the poly(A) tract^{15,16}. In view of the size of this mRNA (~2,000 nucleotides) estimated by blot hybridization analysis of electroplax poly(A) RNA (Fig. 3), it is reasonable to assume that the entire 3' untranslated region is included in the cloned sequence. On the other hand, the 3' untranslated segment so far cloned for the δ -subunit precursor mRNA (213 nucleotides long) contains no polyadenylation signal. Furthermore, this mRNA is ~6,000 nucleotides long (Fig. 3). Thus, it seems likely that the clones isolated for the δ -subunit precursor have been derived from cDNA molecules synthesized by priming at a possible stretch of A residues present upstream of the polyadenylation site.

The individual cDNA clones for the β -subunit precursor used in DNA sequencing exhibit two nucleotide differences, which result in amino acid substitutions in the prepeptide region (Fig. 2a). The cDNA clones for the δ -subunit precursor show one nucleotide difference in the 3' untranslated region (Fig. 2b). The observed nucleotide differences may be due to polymorphism^{17–19} in the respective genes, although possible errors occurring during *in vitro* cDNA synthesis or DNA cloning²⁰ cannot be excluded.

From the mRNA sequences we conclude that the β - and δ -subunit precursors of the *T. californica* AChR consist of 493 and 522 amino acids, respectively. The known amino-terminal sequences of the mature β - and δ -subunits⁶ are preceded by a sequence of 24 (β -subunit) or 21 (δ -subunit) amino acids that resembles the signal peptide of secretory proteins²¹; the prepeptide sequences contain a region rich in hydrophobic amino acids having large side chains in their central portion and terminate in a residue with a small neutral side chain²² (for example, Ala, Gly or Ser). Such a hydrophobic sequence of 24 or 17 amino acids has also been found at the amino-terminal end of the α - and γ -subunit precursors^{12,23}. Thus, these prepeptides are probably involved in the translocation of the receptor subunits into the cytoplasmic membrane. The amino acid compositions of the mature β - and δ -subunits predicted from the mRNA sequences agree fairly well with those determined^{5,11}. Their molecular weights are calculated to be 53,681 (β -subunit) and 57,565 (δ -subunit).

Figure 4 shows the alignment of the amino acid sequences of the α -, β - and δ -subunit precursors of the *T. californica* AChR. The degree of homology is 41, 36 and 41% for the α/β , α/δ and β/δ pairs, respectively; gaps have been counted as one substitution regardless of their length. The degree of homology observed is comparable to that between human α - and β -globins (43%; ref. 24). In addition, 36% (α/β), 36% (α/δ) and 40% (β/δ) of the observed substitutions represent changes among chemically similar amino acids²⁵. These findings strongly suggest that the genes encoding the three subunit precursors have been generated from a common ancestor by gene duplications.

The regions of amino acid sequence homology are distributed



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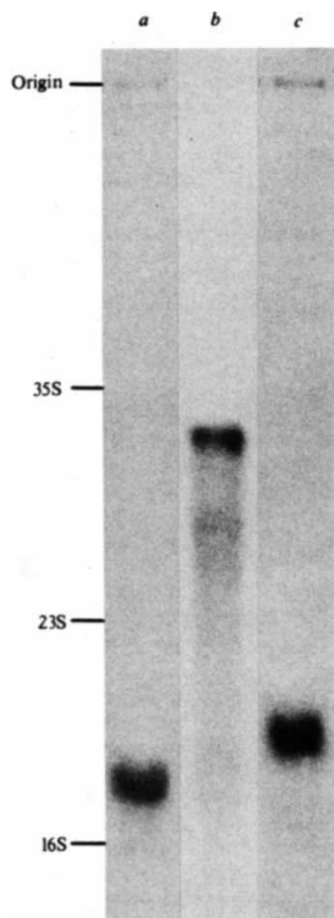


Fig. 3 Autoradiogram of blot hybridization analysis of *T. californica* electroplax RNA using a cDNA probe for the β -subunit precursor (a), δ -subunit precursor (b) and α -subunit precursor (c) of the AChR. Poly(A) RNA (5 μ g) was denatured with 1 M glyoxal and 50% dimethyl sulphoxide³⁸, electrophoresed on a 1.5% agarose gel and transferred to diazobenzyloxymethyl paper³⁹. The hybridization probes used were as follows: a, an equimolar mixture of the *Hinf*I fragment containing residues -15 to 1,246 and the *Hinf*I-*Pvu*II fragment containing residues 1,247-1,607 derived from pACR β 51; b, an equimolar mixture of the *Bst*NI fragments containing residues -171 to 637 and residues 725-1,694 and the *Hind*III fragment containing residues 546-997 derived from pACR δ 4; c, an equimolar mixture of the *Pvu*II fragments containing residues -258 to 1,787 derived from pACR α 44 (ref. 12). The probes were labelled by nick-translation³⁶ with [α -³²P]dCTP. The size markers used were *Escherichia coli* rRNA³⁸ and poliovirus RNA⁴⁰. In a control experiment in which the *Pst*I-cleaved vector plasmid pBR322-SV40 (0.19-0.32) (ref. 13) was used as a probe, no hybridization signals were observed.

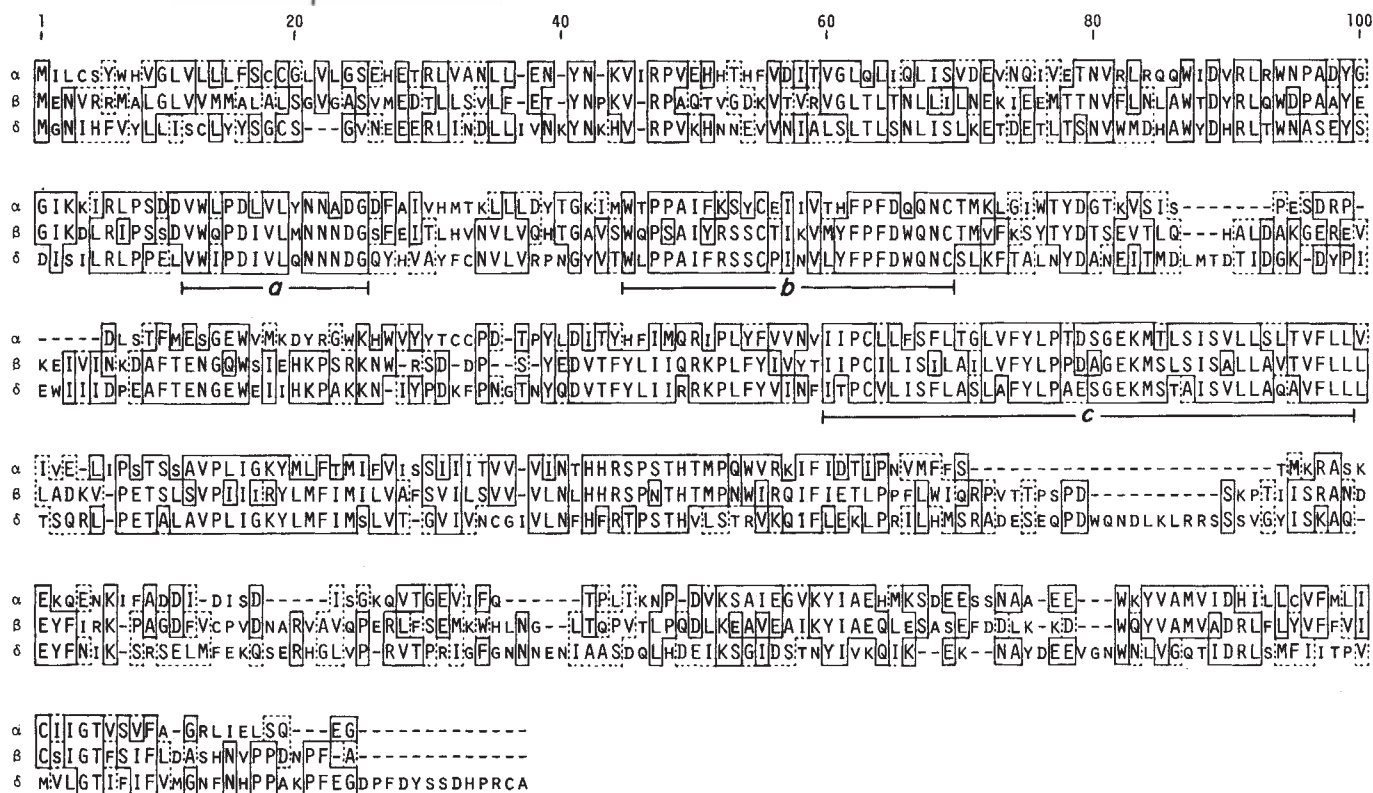


Fig. 4 Alignment of the amino acid sequences of the α -, β - and δ -subunit precursors of the *T. californica* AChR. The amino acid sequence of the α -subunit precursor has been taken from ref. 12. The one-letter amino acid notation is used. Large letters represent sets of identical residues (enclosed with solid lines) and sets of residues considered to be favoured amino acid substitutions (enclosed with dotted lines). Small letters represent the remaining residues. Favoured amino acid substitutions are defined as pairs of residues belonging to one of the following groups: S, T, P, A and G; N, D, E and Q; H, R and K; M, I, L and V; F, Y and W; C (ref. 25). Gaps(-) have been inserted to achieve maximum homology. Regions of highly conserved amino acid sequence are indicated by bars a, b and c.

In addition to the three conserved cysteine residues, the mature β - and δ -subunits contain two (residues 366 and 447) and three (residues 108, 306 and 500) cysteine residues, respectively; residue 500 may be involved in the disulphide bond that links the δ -subunits of adjacent receptor molecules to form a dimer^{31,32} (to be published elsewhere). There are two additional possible sites of *N*-glycosidic linkage in the δ -subunit (asparagine residues 70 and 208).

Computer analysis of the nucleotide and amino acid sequences of the β - and δ -subunit precursors detected no internal homologous units such as those observed for the immunoglobulin polypeptides³³. The codon usage in the δ -subunit precursor mRNA, like that in the α -subunit precursor mRNA¹², exhibits a preference for U (34%) in the third position of the degenerate codons, whereas that in the β -subunit precursor mRNA shows a preference for C (53%) and G (33%). Note also that the δ -subunit precursor mRNA is considerably larger (~6,000 nucleotides) than the α - (~2,300 nucleotides¹²), β - (~2,000 nucleotides) and γ - (~2,100 nucleotides²³) subunit precursor mRNA (Fig. 3).

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Evidence that the insulin secretagogue, β -cell-tropin, is ACTH_{22–39}

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The pituitary neurointermediate lobe of genetically obese (*ob/ob*) mice contains a hormone which stimulates insulin release^{1–3} and which cross-reacts with a -COOH-terminal ACTH antiserum, suggesting that it is related to corticotropin-like intermediate lobe peptide (CLIP), the 18–39 fragment of ACTH^{4–6}. The hormone, which we have called β -cell-tropin⁷, has been shown to be present in the plasma of the *ob/ob* mouse⁸ and to potentiate glucose induced insulin secretion⁹. We have now shown that ACTH_{22–39} prepared by tryptic digestion of human synthetic CLIP behaves similarly on Biogel chromatography and on reverse-phase HPLC to the naturally occurring β -cell-tropin. Furthermore, β -cell-tropin and ACTH_{22–39} have indistinguishable antigenic and insulin releasing properties.

β -Cell-tropin (β -CT), was isolated from obese mouse neurointermediate lobes and purified on Biogel P2–P6 columns and then on reverse phase HPLC as previously described⁹. The biological activity of this material has been tested on the perfused rat pancreas¹⁰ and the results are shown in Fig. 1. Insulin secretion was measured throughout by conventional radioimmunoassay. After stimulation with the test substance, buffer containing a high glucose concentration was introduced in order to confirm the viability of the perfused pancreas preparation. The results indicate that neither two passages on Biogel columns nor further purification with HPLC interfere with the insulin-releasing activity.

Separation of β -CT on reverse phase HPLC is shown in Fig. 2. The amounts of β -CT were measured throughout this investigation by immunoassay using a CLIP antiserum and human CLIP (Bio-products) as standard. The results are expressed as CLIP immunoreactive material (CLIP-IRM)⁸. From the UV absorption and the immunoassay data it was clear that β -CT eluted on the trailing edge of the bovine serum albumin (BSA) peak (Fig. 2a). Preliminary attempts at amino acid analysis of β -CT previously referred to⁹ were discarded, as it was apparent that the peptide was insufficiently purified. Scaling up of the preparation and introducing a second HPLC step allowed separation to give a weak UV absorbing peak following BSA on the trailing edge of another unidentified material (Fig. 2b). The latter material did not cross-react with CLIP antiserum.

At this stage, fast atom bombardment (FAB) mass spectrometry (MS), a new method for the molecular weight and sequence determination of peptides^{11–13} was attempted on the estimated 50–100 pmol of native mouse β -CT. In these initial experiments no result was obtained despite the generation of good quality spectra in control experiments on human CLIP at the 50–100 pmol level (Fig. 3c).

In other work in this laboratory (H.R.M. and M. Panico, unpublished results) we have discovered that arginine-containing peptides exhibit far more intense quasi-molecular ions in FAB-MS than peptides not containing arginine. At the several nanomol level this observation is not critical, and most or all peptides will give a (M+H)⁺ or (M–H)[–] signal in FAB-MS. However, at the sub-nanomol and sub-100 pmol level this phenomenon, which we feel is attributable to the high proton affinity of the guanidino grouping giving a pre-formed (M+H)⁺, can be critical to observing any signals in the FAB spectrum.

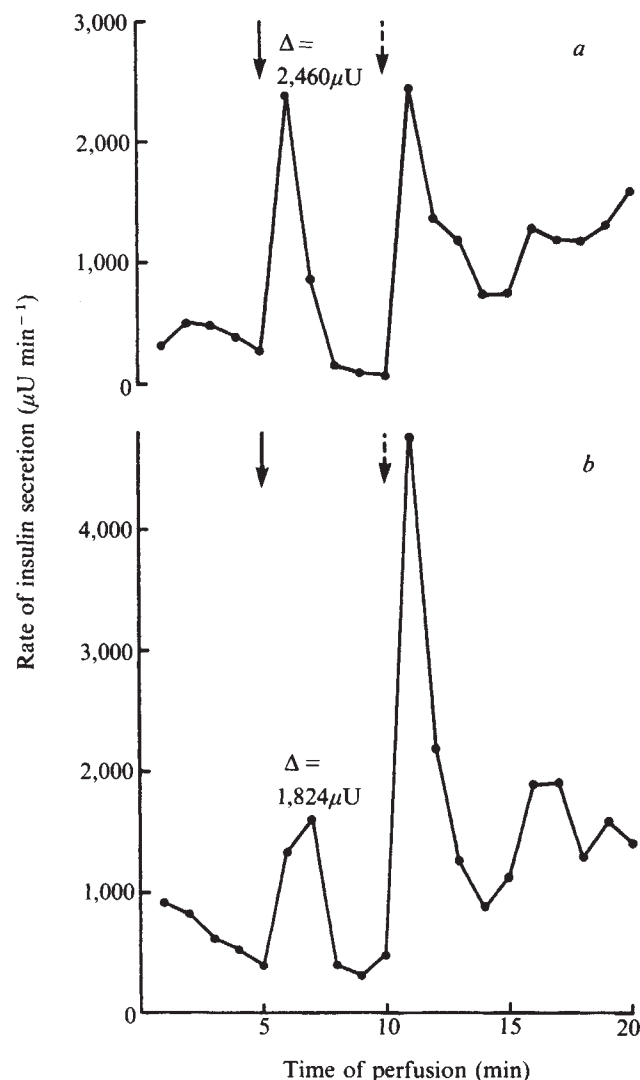


Fig. 1 Stimulation of insulin release by purified β -cell-tropin (β -CT). Pancreata from 250 g male Sprague-Dawley rats were isolated and perfused as previously described¹⁰. Solid arrows indicate start of perfusion with β -CT and broken arrows with glucose 16.6 mmol l^{-1} . *a*, β -CT purified by two separations on Biogel P2-P6 columns⁹, 0.5 ng ml^{-1} CLIP-IRM in perfusion buffer. *b*, As in *a* followed by separation on HPLC⁹, 0.2 ng ml^{-1} CLIP-IRM in perfusion buffer. Δ , Total insulin secreted in excess of basal following stimulation by β -CT.

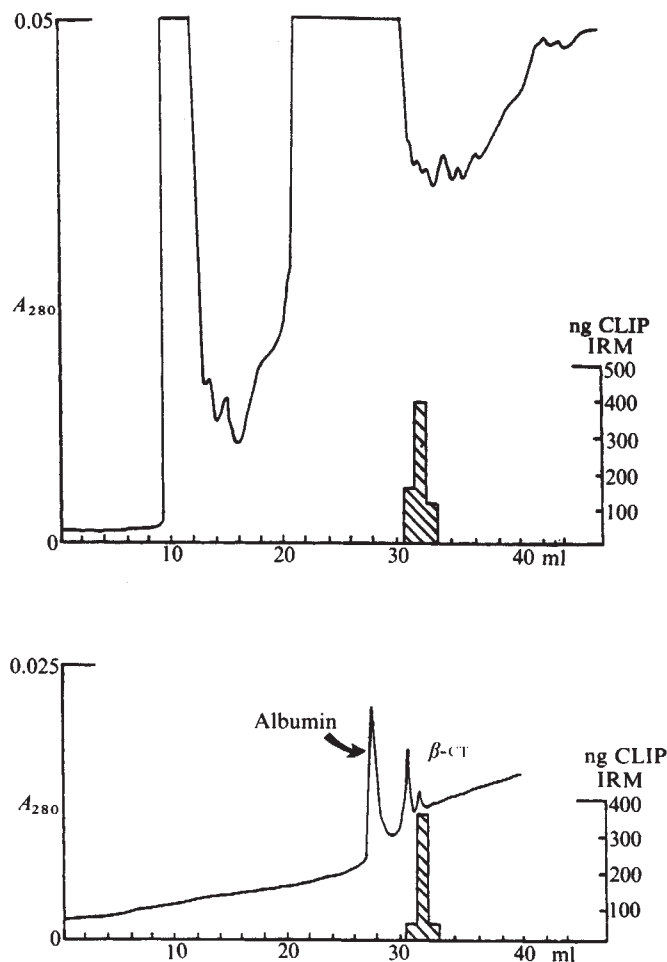


Fig. 2 *a*, Purification of mouse β -CT by reverse phase HPLC. Bioactive material was chromatographed on a μ Bondapak C₁₈ column ($25 \times 0.8 \text{ cm}$) in an *n*-propanol:acetic acid gradient elution system ($10:90-40:60$ over 30 min at 1 ml min^{-1})¹⁸. β -CT eluted as a single immunoreactive peak on the trailing edge of bovine serum albumin, present in 10^4 -fold excess from the preceding Biogel step. *b*, Homogeneous β -CT, free from albumin and other contaminants, was prepared by a second reverse phase HPLC step in identical elution conditions. Immunoreactivity (and biological activity) was associated with a weak A_{280} UV-absorbing peak. HPLC was carried out on a Kontron HPLC system. ▨ Indicates CLIP-IRM.

Thus our failure to obtain FAB-MS signals with mouse β -CT in contrast to CLIP at similar levels led us to argue that if β -CT is a fragment of CLIP it is probably one that lacks an arginine residue, which in turn led us to re-examine the CLIP structure. We deduced that a possible candidate for β -CT would be a product of tryptic cleavage at residues 21-22 of CLIP giving a non-arginine-containing peptide, ACTH₂₂₋₃₉. This peptide would be expected to cross-react with a -COOH-terminal CLIP antiserum and it would have a free amino terminus, as had previously been shown by acetylation of mouse β -CT⁹. Since synthetic mouse CLIP was not available for study, we turned to a commercial preparation of synthetic human CLIP, which differs only in a valine-glycine substitution at residue 26 of ACTH.

The following experiment was therefore designed to test the hypothesis that β -CT is ACTH₂₂₋₃₉: $6 \mu\text{g}$ of human CLIP was treated by mild trypsinization ($1:50$ enzyme:substrate, pH 8.3, 2 h at 37°C) and the digest lyophilized and processed by HPLC chromatography in identical conditions to that for β -CT from the obese mouse (Fig. 2*a*). Apart from the remaining undigested CLIP there was one other major UV absorbing peak (Fig. 3*a*), eluting in an identical position to pituitary β -CT, and cross-reacting with CLIP antiserum. This peptide, when chromatographed on Biogel P2-P6 columns, also co-eluted with pituitary β -CT. The biological activity of synthetic human CLIP and of the peptide isolated from the tryptic digest described above was tested on the perfused rat pancreas. These results (Fig. 4*a*) showed that human CLIP itself had no biological activity

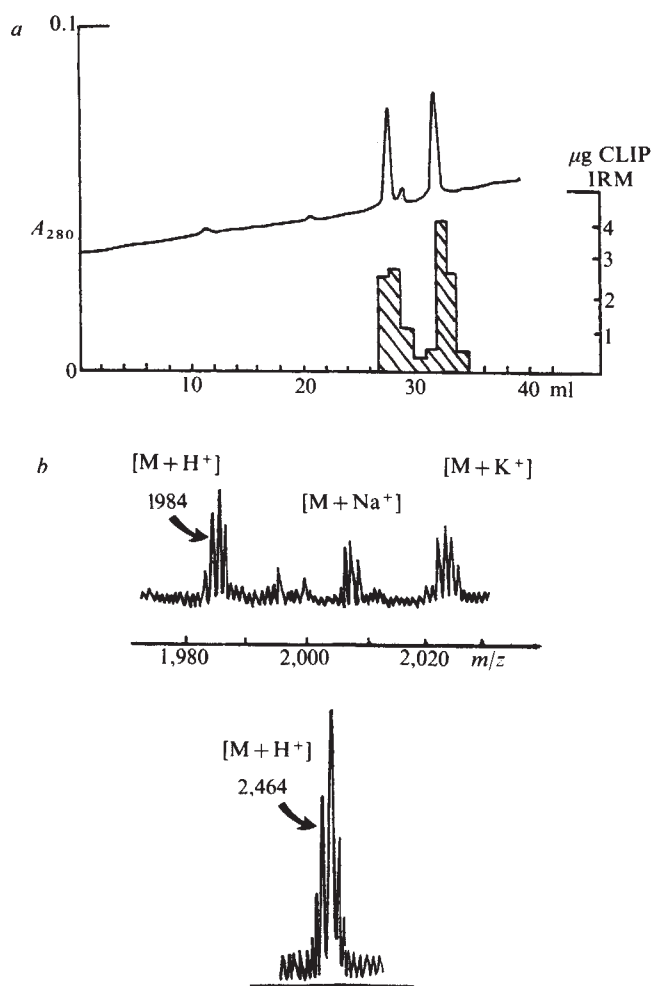


Fig. 3 *a*, Reverse-phase HPLC profile of trypsinized human CLIP (*n*-propanol:acetic acid elution system). CLIP immunoreactivity was associated with two major UV-absorbing peaks. The earlier eluting peak (28 ml) corresponded to undigested CLIP. The second peak (32 ml) had a similar retention time to β -CT, and exhibited the full biological activity of authentic β -CT. *b*, Molecular ion region of the fast atom bombardment (FAB) mass spectrum of human β -CT-like peptide prepared by trypsinization of CLIP. A quasimolecular ion $[M+H]^+$ was observed, from 250 pmol at m/z 1,984, with cationized species at m/z 2,006 $[M+Na]^+$ and m/z 2,022 $[M+K]^+$. At this mass, the natural abundance of ^{13}C (1.1% per carbon atom) results in a more intense '+1' ion at m/z 1,985 (and related signals for cationized species). *c*, Molecular ion region of the FAB mass spectrum of human CLIP (100 pmol) showing an intense quasimolecular ion $[M+H]^+$ at nominal mass m/z 2,464. Note the more abundant ^{13}C -isotope peak at m/z 2,465. Mass spectra were obtained on a VG ZAB-1F mass spectrometer fitted with a high field magnet. Samples were loaded in 30:70 *n*-propanol:acetic acid (5%). Xenon was used as the FAB bombarding species. ▨ Indicates CLIP-IRM.

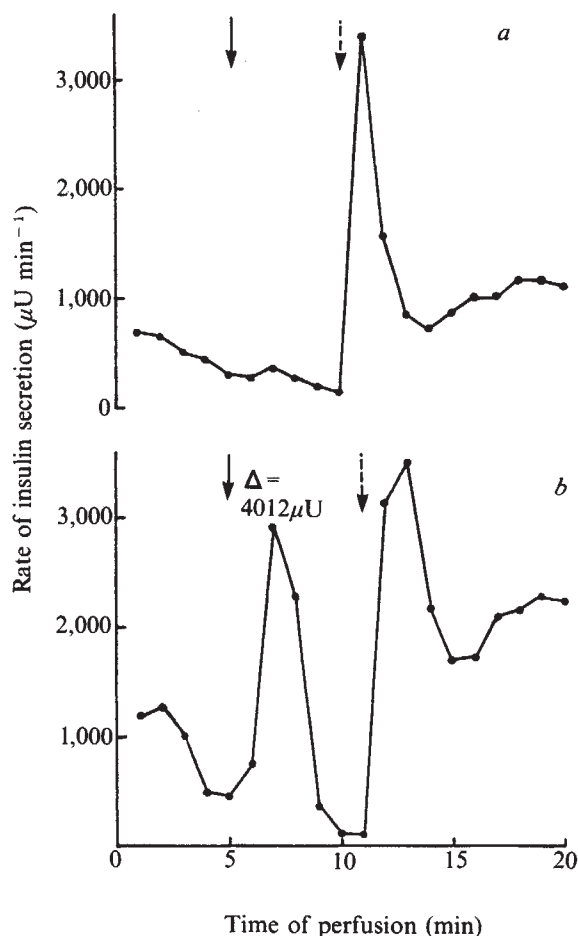


Fig. 4 Influence on insulin release of *a*, human synthetic ACTH₁₈₋₃₉ (CLIP); *b*, ACTH₂₂₋₃₉ (β -CT). ACTH₂₂₋₃₉ was prepared by trypsin digest of human synthetic ACTH₁₈₋₃₉ separated by HPLC and Biogel chromatography⁹. Perfusion was as described in Fig. 1 legend. Solid arrows indicate start of perfusion with peptide, broken arrows with glucose 16.6 mmol l⁻¹. *a*, 1 ng ml⁻¹ CLIP-IRM; *b*, 0.6 ng ml⁻¹ CLIP-IRM.

as previously found with mouse pituitary CLIP⁷, whereas the peptide corresponding to β -CT in elution position gave a similar rapid monophasic stimulation of insulin release (Fig. 4b).

A portion of the tryptic digest of human CLIP (~200 pmol of the active peptide aliquoted from a 3 nmol sample) was analysed by FAB-MS to determine the identity of the CLIP fragment. The spectrum obtained is shown in Fig. 3b and clearly shows the presence of an intense quasimolecular ion at m/z 1,984 ($[M+H]^+$). (Note the more intense ^{13}C isotope peak m/z 1,985 at this mass.) That the signal was not due to some contaminant was proven by 1:1 labelling studies with acetic anhydride: 2H acetic anhydride¹⁴ where the m/z 1,984 singlet shifts to a 1:1 doublet at m/z 2,026/2,029.

A consideration of the CLIP sequence and the experiments carried out allows the identity of the insulin secretagogue to be fixed as ACTH₂₂₋₃₉, a peptide of nominal molecular weight 1,983. All other possible combinations are excluded by the FAB-MS data, even allowing for non-tryptic splits since peptides generated by CLIP 1-n, 2-n, 3-n, 4-n, x-39, y-39, z-39 and so on do not correspond with the FAB-MS data. The identity of the fragment has since been confirmed by amino acid analysis on larger amounts of material. Analysis of aliquots from tubes across the HPLC profile of the tryptic digest showed a concomitant increase and decrease of UV absorbance, CLIP immunoreactivity and amino acid content (Asx₂, Ser, Glx₄, Pro₂, Gly, Ala₃, Val, Leu, Tyr, Phe₂) for the human ' β -CT' peptide.

These findings show that the structure of a human β -CT peptide generated from human CLIP is: Val-Tyr-Pro-Asn-Gly-Ala-Glu-Asp-Glu-Ser-Ala-Glu-Ala-Phe-Pro-Leu-Glu-Phe.

In a further confirmatory study, natural mouse CLIP was isolated from the incubation medium of obese mice neurointermediate lobes⁷. After Biogel chromatography, the sample was trypsinized as described above for human CLIP. The resulting peptide co-eluted with both the naturally occurring mouse β -CT and with the β -CT produced by human CLIP tryptic digestion, on Biogel and HPLC columns. The insulin-releasing activity of this material was also similar to that described with the latter (Fig. 4b).

We were unable to obtain MS data on human ACTH₂₂₋₃₉ at the 200 pmol level after evaporation to dryness and reconstitution of the sample, presumably due to adsorption losses. This correlates well with our inability to obtain MS data on the natural mouse β -CT. Interestingly, such treatment does not affect the ability to obtain spectra from CLIP at the 100 pmol level. To generate sufficient mouse β -CT for definitive structural analysis (1–2 nmol of purified material) will thus require a considerable further effort and this work is in progress.

It is of interest that the insulin-releasing activity shown with ACTH₂₂₋₃₉ (β -CT) is not found with ACTH₁₈₋₃₉ or with a synthetic peptide ACTH₂₅₋₃₉ at comparable concentrations. This suggests that the three N-terminal amino acids of β -CT, that is Val-Tyr-Pro, are essential for biological activity. Acetylation of the free N-terminal amino acid seems to decrease the insulin-releasing activity at comparable concentrations (0.2 ng ml⁻¹).

After the completion of this work we noted that other investigators working on unrelated studies on the structure of ACTH and 'CLIP' have isolated ACTH₂₂₋₃₉ by tryptic digestion^{15,16}. However, no biological activity of this peptide has been described nor has a demonstration that it is a naturally occurring peptide of the pituitary neurointermediate lobe been reported.

The finding that β -CT is present only in very low concentrations in extracts of the pituitary neurointermediate lobes suggests that it is formed by proteolytic breakdown on secretion. Work on the precursor, which could be identical to the proopiomelanocortin precursor, CLIP itself or related molecules with double basic residues before the β -CT sequence is under investigation. It has been shown that the insulin-releasing activity of the pituitary is high in the *ob/ob* mouse and other animal models of obesity^{5,10}. It is perhaps significant that other peptides derived from the proopiomelanocortin precursor in the neurointermediate lobe have also been shown to be elevated in obesity¹⁷.

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Detection of hepatitis B surface antigen using time-resolved fluoroimmunoassay

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Conventional fluoroimmunoassay (FIA) methods based on various fluorescence principles have not achieved the sensitivity of radioimmunoassay (RIA) mainly because of problems of background fluorescence arising, for example, from the biological specimen. We now describe an immunoassay of hepatitis B surface antigen (HBsAg) based on time-resolved (TR) fluorescence¹ using a lanthanide as label. The assay initiates the development of a new generation of immunoassays. The fluorescence intensity is measured after a selected delay time which almost completely eliminates background fluorescence, which has a fast decay time. The excitation is performed with a flashing light source. The molecules with a long fluorescent lifetime consist of chelates of rare earth metals (Eu, Tb, Sm, Dy). They absorb strongly the excitation radiation and transfer the energy to the chelated central atom which in turn produces an emission spectrum characteristic of the lanthanide used. A long Stokes' shift (>270 nm) helps to reduce the background in the emission region of the chelate and thus optimizes measurement of the relevant fluorescence. The present TR-FIA uses 2-naphthyltrifluoroacetone as chelating agent because it creates an intense fluorescence with the rare earth metals. Synergistic agents such as trioctylphosphineoxide further enhance the fluorescence of the chelate. Depending on the instrumentation used for measuring time-resolved fluorescence and the conditions used for chelate formation, lanthanides can be detected at 10⁻¹²–10⁻¹⁴ M concentrations^{1,2}.

The lanthanide must be firmly bound to the antibody, preferably without affecting the immunological activity of the label-antibody conjugate. The complex should, in addition, be kinetically stable during the immunoassay. This is achieved by the use of EDTA, which has extremely high binding constants for lanthanides. An aminophenyl derivative of EDTA has been synthesized which allows linkage to proteins. The amino group is activated by diazotization and then reacted with tyrosine and histidine residues in the protein to be labelled. The conjugation reaction must be strictly controlled to preserve the biological activity of the antibodies.

Hepatitis B surface antigen was chosen as a model system to test the performance of TR-FIA. Hepatitis B virus (HBV) infections are a frequent cause of serious disease in exposed persons^{3,4}, where the virus can produce up to 10¹³ virus-antigen-bearing particles per ml of host plasma. The virus is spread by direct contact with contaminated material, of which transfused blood constitutes one potential risk. Thus, assays for the various antigens and antibodies associated with HBV infection in man are of established value in patient diagnosis and assessment as well as in the identification of blood donations capable of transmitting infection.

The most common immunological marker of HBV infection is HBsAg, and the various methods for its detection have been classified according to their sensitivity and historical development as first, second and third generation methods⁵. An assay has third generation performance only if it detects all of a number of weakly positive samples issued by the US Bureau of Biologics, Food and Drug Administration. Solid-phase RIA and enzyme immunoassay methods fulfil this criterion. Current

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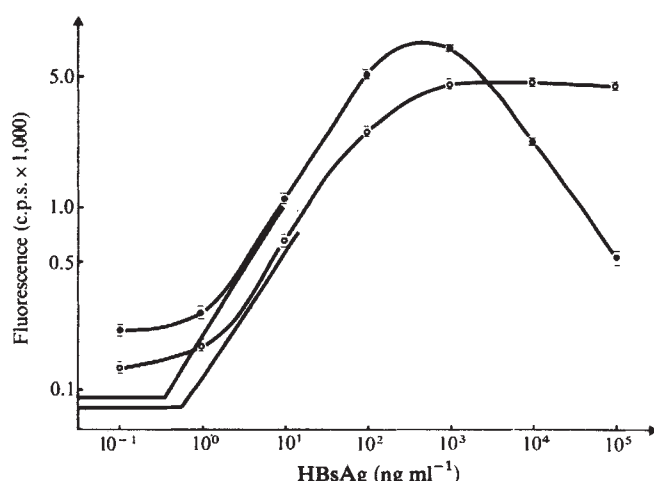


Fig. 1 Standard TR-FIA curves of purified HBsAg (ad-type) diluted in negative plasma. The assay was performed with a two-step (○) and one-step (●) incubation procedure. Each point represents the mean of three assays. The horizontal lines give the signal level of the negative controls and they are intersected by the line obtained by subtracting the negative control from the standard curve.

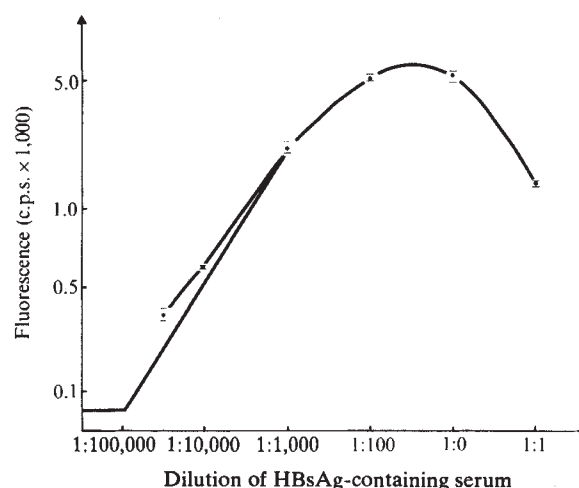


Fig. 2 Dilution curve for a plasma specimen containing HBsAg obtained with the one-incubation TR-FIA. The meaning of the horizontal and intersecting line is as stated in Fig. 1. Each point represents the mean of three assays.

solid-phase RIAs detect $\sim 0.5 \text{ ng ml}^{-1}$ of HBsAg protein and have been the most sensitive and reliable methods available so far. Notwithstanding, due to certain drawbacks of RIA—radiation hazard and the relatively short half life of certain isotopes—attempts have been made to replace radioisotopes with sensitive non-isotopic labels.

The TR-FIA of HBsAg described here can be performed as either a two- or one-step assay. In both assays monoclonal antibodies obtained from the Finnish Red Cross Blood Transfusion Service were used. The ascites fluid was pooled from two different clones. The catching and the labelled antibodies were the same. In the two-step assay the immunoglobulin fraction of the pooled ascites fluid was used to coat polystyrene tubes. The coating was performed in 0.2 M borate-buffered saline (250 μl , 2 μg protein), pH 9.3, overnight at room temperature and the surface was saturated with 0.5% bovine serum albumin in 0.05 M Tris-HCl-buffered saline, pH 7.7, containing 0.05% NaN_3 , for 1 h at room temperature. The labelled antibody was prepared by conjugating a diazotized aminophenyl-EDTA-europium complex to affinity-purified HBsAg antibodies from the pooled ascites fluid. The conjugation was carried out by careful neutralization of the diazotized material to pH 5–6 with saturated sodium hydrogen carbonate and by adding this reagent to the antibody solution in 50–100 fold molar excess. The pH was adjusted to 8.5–9.5 by the addition of sodium hydroxide and the conjugation was carried out overnight at $+4^\circ\text{C}$. A Sephadex G-50 column (1.5 $\times$ 25 cm), eluted with 0.05 M Tris-HCl buffer, pH 7.7, containing 9 g l^{-1} NaCl and 0.05% NaN_3 , was used to purify the antibody from excess label. The plasma specimen (200 μl) was incubated for 2 h at 40°C in a coated tube. After three washings with saline solution the second incubation with labelled antibody (10 ng per tube) was performed for 1 h at $+40^\circ\text{C}$ in 200 μl 0.05 M Tris-HCl-buffered saline, pH 7.7, containing 0.05% NaN_3 and 5% of both normal human and sheep serum. The tube was washed three times with saline solution and the Eu fluorescence was measured for 1 s in a TR-fluorometer. The fluorometer uses a xenon flashtube as a pulsed light source and the time-resolving operation is controlled by control pulses from a clock pulse generator. The length of a flash is 0.5 μs and the delay time from the flash trigger pulse to the start of scaling is 50 μs ; the fast scaler gate open-cycle is 250 μs . The excitation and emission wavelengths are 330 and 613 nm, respectively. The total measurement time per sample is only 1 s. A typical HBsAg dilution curve obtained with the two-step sandwich assay using a purified HBsAg preparation⁶ is shown in Fig. 1. The sensitivity

of the assay is $<1 \text{ ng ml}^{-1}$ when defined as twice the signal level of the negative control.

The two-step procedure takes at least 3 h to produce the result, but urgent results are required quite often. This has previously led to a compromise which increases the risk of HBV infection. Solid-phase RIA tests can be used with shortened incubation times (for example, 45 min), with a two- to fourfold loss of sensitivity. Hence, the development of a single-step solid-phase TR-FIA of HBsAg using monoclonal antibodies became a challenge. The plasma specimen (100 μl) was pipetted to the coated tube simultaneously with the labelled antibody. The same coating and labelling procedure was used as for the two-step assay. The tube was incubated for 2 h at 40°C and washed three times with saline. The Eu fluorescence was developed for 20 min in the presence of 2-naphthyltrifluoroacetone (15 μM) and trioctylphosphineoxide (50 μM) and measured for 1 s. The result of the one-step assay using the same specimen as in the two-step procedure is shown in Fig. 2. The test is even more sensitive than the conventional method with two incubations. The detection limit for HBsAg is $\sim 0.5 \text{ ng ml}^{-1}$ if twice the signal level of the negative control is used as a limit. Reduction of the incubation time to 15 min resulted in only a fourfold loss in sensitivity.

A dilution curve using the single-step procedure for a typical HBsAg positive specimen is shown in Fig. 2. The test is still positive at a 1:20,000 dilution. The actual HBsAg content of the specimen has been estimated to 20 $\mu\text{g ml}^{-1}$, which implies that the sensitivity of the assay was at least 0.2 ng ml^{-1} if defined as twice the negative control.

The increased sensitivity obtained in the one-step procedure presumably depends on the increased binding of the labelled antibody to the antigen in the liquid phase. Consequently, the number of labelled antibody molecules bound per antigen is increased, but this does not yet limit the binding of the antigen to the solid phase antibody. A more sensitive assay of low HBsAg concentration is thus achieved. The decrease in the signal at very high HBsAg concentration is due to the binding of labelled antibody to the excess of the liquid phase antigen that will be washed away. Hence, the reduction of the number of labelled antibodies available per antigen will decrease the signal when the HBsAg concentration reaches a certain level. A high antigen concentration in the sample will result in an asymptotic convergence of the signal towards that obtained for negative controls, but according to the present experience of the one-step procedure a specimen containing 10^{13} HBsAg

particles ($100 \mu\text{g ml}^{-1}$) still gives a positive to negative ratio of 7, which is well above the limit.

The results show that the TR-FIA using Eu-labelled monoclonal antibodies is a simple one-step procedure for the detection of HBsAg which exceeds RIA in sensitivity, at the same time avoiding all the disadvantages of the use of isotopes. The assay could simplify the large-scale screening of blood donors, and the sensitivity of the test should decrease the number of false negative results and thus reduce the number of post-transfusion HBV infections. A more sensitive technique will also assist HBsAg detection earlier during the incubation period and in the diagnosis of chronic HBV infection, in which low levels of HBsAg are present. In addition, the development of TR-FIA offers considerable potential for improvements in the whole field of immunoassay technology.

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Nucleotide sequence of cassava latent virus DNA

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Only two groups of plant viruses, the caulimoviruses^{1,2} and the geminiviruses³, are known to contain a genome of DNA. Unlike that of the caulimoviruses, the genome of the geminiviruses is composed of single-stranded, covalently-closed circles of DNA. There is evidence that the geminiviruses, specifically bean golden mosaic virus⁴ and tomato golden mosaic virus⁵, have a genome composed of two similar-sized circles of DNA, and the molecular cloning of both components from tomato golden mosaic virus has recently been reported⁶. No information is available, however, concerning the protein coding capacity of the genome, the possible modes of RNA transcription and DNA replication and the assembly of mature components into the characteristic geminate particles. We report here on the derivation of the nucleotide sequence of the geminivirus, cassava latent virus (CLV), as a fundamental step towards the investigation of the mode of replication of this group of viruses. Results show that CLV DNA comprises two similar-sized molecular species with a common region encompassing almost 200 nucleotides. The organization of the genome is discussed.

With the presently available rapid sequencing techniques, it was considered unnecessary to derive a detailed restriction enzyme map of the genome before initiating the investigation of the sequence. Furthermore, it was assumed that the genome of CLV would mirror those of other geminiviruses so far examined and have a bipartite genome^{4,5}. This being the case, exhaustive sequencing of the viral DNA should lead to the accommodation of all derived data into two circular structures. This was found to be the case.

The strategy adopted for the elucidation of the sequence is outlined in Fig. 1 legend. As the CLV genome is single stranded, it was necessary to generate a second, complementary strand before cloning the material into M13 vectors. The sequence GGAATTCC, found within part of the genome, enabled us to use a double-stranded octanucleotide of the same sequence to prime DNA polymerase-directed second-strand synthesis on the viral DNA template. Although this octanucleotide sequence appears only once, at position 1,867 in DNA 1, cloned material from both DNAs 1 and 2 was generated by this method. Presumably, the sequence GGAATTCA at position 1,527 in DNA 2 bound the primer sufficiently well to allow primer

extension even in the presence of the terminal mismatch. By the combination of the various approaches described in Fig. 1 legend, 90% and 80% of the sequences of DNAs 1 and 2, respectively, were determined in both orientations.

The sequences of the CLV DNAs are shown in Fig. 1, and indicate that the viral DNA does, in fact, contain two sequences of similar length, DNAs 1 and 2, containing 2,779 and 2,724 nucleotides, respectively (the larger of the two DNAs was arbitrarily designated DNA 1). This size difference of less than 2% will account for the failure to resolve the molecules by gel electrophoresis⁷.

Although the entire sequence was established from a single preparation of virus, a number of nucleotide variations were found within the fragments that were cloned in M13; these are shown in Fig. 1 as an alternative nucleotide below the sequence. As the virus preparation was not derived from a local lesion isolate, it might be expected to contain a population of slightly varying molecules.

There is a region of almost 200 nucleotides common to both DNAs, the 5' extremities of these regions having been arbitrarily designated as nucleotide 1 in Fig. 1. Figure 2 compares the homologous sequences. In addition to small repeated or near-repeated sequences, the region shows one potentially very stable hairpin structure between nucleotides 133 and 165, the loop of which is composed almost entirely of A and T residues. The purpose of a region common to both molecules is unknown but it may be speculated that it serves as a recognition site during the process of virus multiplication, possibly containing transcription promoters (see below) and the origin of DNA replication. This is the only region showing extensive homology between the two DNA molecules.

To investigate the potential coding capacity of the genome, the sequences were screened in all three reading frames for open regions. The results of this, both for the viral DNA sequences as presented in Fig. 1 and for their complement, are summarized in Fig. 3. Based on these data, and bearing in mind that no information is available concerning possible splicing and polypeptide maturation events, a tentative proposal for a number of virus-specific proteins is suggested in Fig. 4. It was assumed that the first ATG triplet in each open region would initiate protein synthesis and only those open regions with a potential coding capacity of molecular weight (M_r) >10,000 are included in the figure. Figure 4 shows that, when read in the virion DNA sense, the sequence of DNA 1 can code for overlapping proteins of M_r s 30,100 and 12,400. However, when read in the opposite sense, ~85% of the sequence may be involved in protein coding to give products of M_r s 40,200, 27,000, 15,600, 15,500, 15,100, 13,500 and 10,600. All sequences involved lie outside the region common to the two DNAs.

The situation for DNA 2 seems to be slightly less complex. DNA 2 is capable of coding for a protein of M_r 29,200 and a smaller protein of M_r 13,500 when read in the virion DNA sense, and a protein of M_r 33,600, overlapping with the putative M_r 13,500 protein, when read in the opposite sense. The open reading regions of the two large putative proteins do not overlap, being separated by a short A + T-rich sequence. Together, the two major putative proteins utilize ~60% of the available sequence and, as seen for DNA 1, all potential coding regions lie outside the region common to both viral DNAs.

The data concerning the open reading regions of DNA 2 strongly suggest that transcription of both the viral DNA and its complement takes place, implying the production of a double-stranded intermediate. A double-stranded viral DNA structure, isolated from plant material infected with bean golden mosaic virus, has been shown to be infectious, suggesting that it is competent for RNA transcription⁸. Additional evidence for the bidirectional nature of transcription of the genome is provided by an analysis of the amino acid content of each putative protein. The amino acid composition of the CLV coat protein (M. Short, personal communication) shows a close correlation with that of the putative 30,100 M_r protein of DNA

Fig. 1 The nucleotide sequence of DNA 1 (left) and DNA 2 (right). CLV (West Kenyan isolate 844) was propagated and the DNA isolated from purified virions as described previously¹². The initial strategy adopted to accumulate sequence data was to digest the viral genome with restriction enzymes *HaeIII* and *HhaI*, known to cut single-stranded DNA^{13,14}. The products of digestion were terminally labelled at either the 5' (ref. 15) or 3' (ref. 16) termini and sequences established using the chemical degradation technique of Maxam and Gilbert¹⁵. The identification of the octanucleotide GGAATCC within the derived sequences allowed the use of double-stranded DNA of the same sequence (molecular linker recognized by *EcoRI*) to prime DNA polymerase-directed second-strand synthesis using the viral DNA as the template, essentially as described by Hong¹⁷. The resulting double-stranded DNA was digested with restriction enzymes *BamHI*, *BglII*, *EcoRI*, *HindIII*, *MboI*, *MspI*, *PstI* or *TaqI* and fragments cloned in the appropriate M13 vector¹⁸. After selection of recombinants and isolation of M13 DNA, sequences of the inserts were established using the dideoxy-termination procedure¹⁹, using as a primer a synthetic deoxyribonucleotide 17 nucleotides in length²⁰. At positions where sequence data remained ambiguous, the replicative form of appropriate M13 clones was isolated, and the inserted DNA excised with a suitable restriction enzyme and purified by sucrose gradient centrifugation. Sequence analysis of enzyme digests of the inserts was by the method of Maxam and Gilbert¹⁵.

10	20	30	40	50	60	10	20	30	40	50	60
CTCAACTGGA	BACACTCTTG	AGCATCTCCT	CCTATTAAIT	GGAGACATTA	TATAGBTGTC	CTCAACTGGA	BACACTCTTG	AGCATCTCCT	CCTATTAAIT	GGAGACATTA	TATAGBTGTC
70	80	90	100	110	120	70	80	90	100	110	120
TCTAAATGCG	ATTCTGTGAA	TAAAGTGAAC	TTTAATTGGA	ATTAAGAAGC	TCAAAGBCT	TCTAAAGBCT	ATTCTGTGAA	TAAAGTGAAC	TTTAATTGGA	ATTAAGAAGC	TCAAAGBCT
130	140	150	160	170	180	130	140	150	160	170	180
CAGAACACCC	AAGGGGCCAA	CCGTATAATA	TTACCGGTGT	CCCCGCCCCC	CTTTAATGTG	CAGAACACCC	AAGGGGCCAA	CCGTATAATA	TTACCGGTGT	CCCCGCCCCC	CTTTAATGTG
190	200	210	220	230	240	190	200	210	220	230	240
GTCCGCCBGC	ACTACTTATG	TCGGCCAACT	ATGATGTAGC	TTTAAGGTTT	ATGTATTAGT	GTCCGCCBGC	ACTACTTATG	TCGGCCAACT	ATGATGTAGC	TTTAAGGTTT	ATGTATTAGT
250	260	270	280	290	300	250	260	270	280	290	300
GTGGGCCACG	TATATACTGT	CAGGCGAAGT	TGTGGCTAGT	GCACATATGT	GGATCCACTG	GTGGGCCACG	TATATACTGT	CAGGCGAAGT	TGTGGCTAGT	GCACATATGT	GGATCCACTG
310	320	330	340	350	360	310	320	330	340	350	360
GTGAATGAGT	TTCCGACTCT	GTGCTATGCG	CTTAGGTGTA	TGCTGGCAAT	TAAATATTBT	GTGAATGAGT	TTCCGACTCT	GTGCTATGCG	CTTAGGTGTA	TGCTGGCAAT	TAAATATTBT
370	380	390	400	410	420	370	380	390	400	410	420
CAGGCTTAGG	AGGATACATA	CGAGCCCACT	ACGTTGGGCC	ACGAACCTGT	GAGGGATCTA	CAGGCTTAGG	AGGATACATA	CGAGCCCACT	ACGTTGGGCC	ACGAACCTGT	GAGGGATCTA
430	440	450	460	470	480	430	440	450	460	470	480
GTCTCACTTA	TCAGGGCTCG	TAAATTATGT	GAAAGCAGCA	GGAGATCTGA	TCATTTCACG	GTCTCACTTA	TCAGGGCTCG	TAAATTATGT	GAAAGCAGCA	GGAGATCTGA	TCATTTCACG
490	500	510	520	530	540	490	500	510	520	530	540
TCCAGGATCC	AAGGTTCTGT	GAAAGCTGAA	CTTCGACAGC	CCATACAGGA	ACCGTGTACT	TCCAGGATCC	AAGGTTCTGT	GAAAGCTGAA	CTTCGACAGC	CCATACAGGA	ACCGTGTACT
550	560	570	580	590	600	550	560	570	580	590	600
TGCCCCCACT	GTCCACGTGA	CAATTCGAAA	ACGGGCTGGT	GTGACAGGCG	CCATGTACAG	TGCCCCCACT	GTCCACGTGA	CAATTCGAAA	ACGGGCTGGT	GTGACAGGCG	CCATGTACAG
610	620	630	640	650	660	610	620	630	640	650	660
AAGGCCCAAC	ATGTACAGGA	TGTATAGAAG	CCGACAGCTA	CCTAGGGGCT	GTGAAGGCCG	AAGGCCCAAC	ATGTACAGGA	TGTATAGAAG	CCGACAGCTA	CCTAGGGGCT	GTGAAGGCCG
670	680	690	700	710	720	670	680	690	700	710	720
ATGTAGGCTG	CAGTCTGTGT	AGCAGAGGGA	TGATBTGAAG	CACCTTGCTA	TCTGTAAAGT	ATGTAGGCTG	CAGTCTGTGT	AGCAGAGGGA	TGATBTGAAG	CACCTTGCTA	TCTGTAAAGT
730	740	750	760	770	780	730	740	750	760	770	780
DATTAGTGAT	GTGACGCGTG	GGCCTGGGCT	GACACAGAGG	GTGCGAAGA	GGTTTGTAT	DATTAGTGAT	GTGACGCGTG	GGCCTGGGCT	GACACAGAGG	GTGCGAAGA	GGTTTGTAT
790	800	810	820	830	840	790	800	810	820	830	840
CAGTCTCAT	TACATTCTGT	GTAACTCTGT	CGTGGTGAAG	ACTATTAGA	AGCAAACTCA	CAGTCTCAT	TACATTCTGT	GTAACTCTGT	CGTGGTGAAG	ACTATTAGA	AGCAAACTCA
850	860	870	880	890	900	850	860	870	880	890	900
CAGTAAAT	GTGATTTTGT	ACCTCTCTAG	GGATAGAAGG	CCGATAGGCA	ATGCGCCCA	CAGTAAAT	GTGATTTTGT	ACCTCTCTAG	GGATAGAAGG	CCGATAGGCA	ATGCGCCCA
910	920	930	940	950	960	910	920	930	940	950	960
AGACTTCGCG	CAGATATTGA	ACATGTTTGA	TAAAGTACCC	AGTACTGCGA	CAATTAAGAA	AGACTTCGCG	CAGATATTGA	ACATGTTTGA	TAAAGTACCC	AGTACTGCGA	CAATTAAGAA
970	980	990	1000	1010	1020	970	980	990	1000	1010	1020
CGATTTCGAG	DATAGGTTTC	AGGTTGTGAG	GAATTTTCAT	CCGACTGTTC	TGTGTGTTCC	CGATTTCGAG	DATAGGTTTC	AGGTTGTGAG	GAATTTTCAT	CCGACTGTTC	TGTGTGTTCC
1030	1040	1050	1060	1070	1080	1030	1040	1050	1060	1070	1080
ATATGCGATG	AGGAGGACAG	CGTGTGTGAA	AGGTTTTCAT	AGGTTGATTC	ATCAGTGTTC	ATATGCGATG	AGGAGGACAG	CGTGTGTGAA	AGGTTTTCAT	AGGTTGATTC	ATCAGTGTTC
1090	1100	1110	1120	1130	1140	1090	1100	1110	1120	1130	1140
ATACATCACT	CAGGAGGACG	GGAGATATGA	GAATCAGACA	GAGATGTTCT	TGCTTCTGTA	ATACATCACT	CAGGAGGACG	GGAGATATGA	GAATCAGACA	GAGATGTTCT	TGCTTCTGTA
1150	1160	1170	1180	1190	1200	1150	1160	1170	1180	1190	1200
CATGCGATGT	ACTATGCTCT	CCATCTCTGT	ATATGCGAGC	TTGAAATATC	GTATATCTTC	CATGCGATGT	ACTATGCTCT	CCATCTCTGT	ATATGCGAGC	TTGAAATATC	GTATATCTTC
1210	1220	1230	1240	1250	1260	1210	1220	1230	1240	1250	1260
CTATGCGATG	ATTGCGCAAT	AATAAATCAT	GAATTTTAT	TCATGAGCTA	ACTGTGCTTC	CTATGCGATG	ATTGCGCAAT	AATAAATCAT	GAATTTTAT	TCATGAGCTA	ACTGTGCTTC
1270	1280	1290	1300	1310	1320	1270	1280	1290	1300	1310	1320
AATAGTGTG	GCATATACAT	TGAAACAAAC	ATGATCAGCA	GCCTCTATTA	CATGTATTAAT	AATAGTGTG	GCATATACAT	TGAAACAAAC	ATGATCAGCA	GCCTCTATTA	CATGTATTAAT
1330	1340	1350	1360	1370	1380	1330	1340	1350	1360	1370	1380
TGAGTAAACA	CCATATATTAT	CCAGTATATT	AATCTCTGTC	TATCTAAGA	CCCTTAAGAA	TGAGTAAACA	CCATATATTAT	CCAGTATATT	AATCTCTGTC	TATCTAAGA	CCCTTAAGAA
1390	1400	1410	1420	1430	1440	1390	1400	1410	1420	1430	1440
AAGACGATC	TGAGGCTGTA	AGGTTGTCCA	GATCTGGAGG	TTGAGAAACG	ATTGTGTAAT	AAGACGATC	TGAGGCTGTA	AGGTTGTCCA	GATCTGGAGG	TTGAGAAACG	ATTGTGTAAT
1450	1460	1470	1480	1490	1500	1450	1460	1470	1480	1490	1500
CCGACGCTC	TTCTCCAGGT	TGTGATTGAA	TGCAAGCTGG	ACTGTATATGA	TGTCTGGTTC	CCGACGCTC	TTCTCCAGGT	TGTGATTGAA	TGCAAGCTGG	ACTGTATATGA	TGTCTGGTTC
1510	1520	1530	1540	1550	1560	1510	1520	1530	1540	1550	1560
CAGCAGGAAT	GGTCTGTGTT	GGTCTGTGTT	GATTTGGAAT	TACAGGAGAT	TGTATTATTC	CAGCAGGAAT	GGTCTGTGTT	GGTCTGTGTT	GATTTGGAAT	TACAGGAGAT	TGTATTATTC
1570	1580	1590	1600	1610	1620	1570	1580	1590	1600	1610	1620
CCAGTATATC	ACGCGATCTA	TTGCTTGAGG	AGCAGTATGT	ACTTCCCTGC	TGCTTAATAT	CCAGTATATC	ACGCGATCTA	TTGCTTGAGG	AGCAGTATGT	ACTTCCCTGC	TGCTTAATAT
1630	1640	1650	1660	1670	1680	1630	1640	1650	1660	1670	1680
CATGATTTGA	CCAGTGTGTA	TGAGGATTAAT	ATGACATCTC	ACACAGAGGA	TCCACTCTCC	CATGATTTGA	CCAGTGTGTA	TGAGGATTAAT	ATGACATCTC	ACACAGAGGA	TCCACTCTCC
1690	1700	1710	1720	1730	1740	1690	1700	1710	1720	1730	1740
TACGCGGATG	GGTCTGCTCT	TTGACTGTCT	TGTGAGTACG	TTTGAATGGA	CCGATGATAG	TACGCGGATG	GGTCTGCTCT	TTGACTGTCT	TGTGAGTACG	TTTGAATGGA	CCGATGATAG
1750	1760	1770	1780	1790	1800	1750	1760	1770	1780	1790	1800
AGTGGTCTG	TGAGGATGTA	GAAGATTTGA	TTCTTTAATG	CCGACGCTCT	TACGCTCTCT	AGTGGTCTG	TGAGGATGTA	GAAGATTTGA	TTCTTTAATG	CCGACGCTCT	TACGCTCTCT
1810	1820	1830	1840	1850	1860	1810	1820	1830	1840	1850	1860
TGCTTTTCT	CGGCTAGGAA	CTCTTTATAG	GACAGAGGAT	GTCTTGATGT	CAGAGAGGAA	TGCTTTTCT	CGGCTAGGAA	CTCTTTATAG	GACAGAGGAT	GTCTTGATGT	CAGAGAGGAA
1870	1880	1890	1900	1910	1920	1870	1880	1890	1900	1910	1920
ATAGTGGGAA	TTCCACCTTT	AATTTGAAGC	GGTTTCCGCT	ATTTCGTGTT	GGACTGGACG	ATAGTGGGAA	TTCCACCTTT	AATTTGAAGC	GGTTTCCGCT	ATTTCGTGTT	GGACTGGACG
1930	1940	1950	1960	1970	1980	1930	1940	1950	1960	1970	1980
TTCCCTCTGG	ACCCCATGAA	TTCTTTAAGG	TGCTTTTAGT	AGTGGGATCT	GACGCTATCA	TTCCCTCTGG	ACCCCATGAA	TTCTTTAAGG	TGCTTTTAGT	AGTGGGATCT	GACGCTATCA
1990	2000	2010	2020	2030	2040	1990	2000	2010	2020	2030	2040
ATGACCTGTT	ACGAGCGACG	ATTATTTGAG	ACCTTTGAGC	TAAAGTGTGA	GTGCTCACAC	ATGACCTGTT	ACGAGCGACG	ATTATTTGAG	ACCTTTGAGC	TAAAGTGTGA	GTGCTCACAC
2050	2060	2070	2080	2090	2100	2050	2060	2070	2080	2090	2100
AGGTAATATT	GTGGGCTCAA	AGATCTGCCC	CATATCTGCT	TCCCTTGCTT	GCATATCACT	AGGTAATATT	GTGGGCTCAA	AGATCTGCCC	CATATCTGCT	TCCCTTGCTT	GCATATCACT
2110	2120	2130	2140	2150	2160	2110	2120	2130	2140	2150	2160
TCTATGACAA	TACTATTAGG	TCTCCATGCG	CGGCGAGCGG	AATCCCTTAC	ATTATCAGCG	TCTATGACAA	TACTATTAGG	TCTCCATGCG	CGGCGAGCGG	AATCCCTTAC	ATTATCAGCG
2170	2180	2190	2200	2210	2220	2170	2180	2190	2200	2210	2220
ACCCATCTTT	CAATTTTACG	AGGAACCTGG	TCAAAGGAGG	AACATGGGAA	GGGAGAAACA	ACCCATCTTT	CAATTTTACG	AGGAACCTGG	TCAAAGGAGG	AACATGGGAA	GGGAGAAACA
2230	2240	2250	2260	2270	2280	2230	2240	2250	2260	2270	2280
TAAAGGAGCT	GTGGCTCTGT	GAAATCTCTA	TCTAAATATC	TATTTAGATT	ATGAACCTGA	TAAAGGAGCT	GTGGCTCTGT	GAAATCTCTA	TCTAAATATC	TATTTAGATT	ATGAACCTGA
2290	2300	2310	2320	2330	2340	2290	2300	2310	2320	2330	2340
AGTACAAAGT	CTTTTGGGAC	TAACTTCTCA	ATGACATTTA	GAGCTTCTGA	CTTACTGCG	AGTACAAAGT	CTTTTGGGAC	TAACTTCTCA	ATGACATTTA	GAGCTTCTGA	CTTACTGCG
2350	2360	2370	2380	2390	2400	2350	2360	2370	2380	2390	2400
CTTTTGGGAC	CTTTTGGGAC	AGCATCTCTG	GTGATGTTT	GACCGCTCTT	AGCAGATCTG	CTTTTGGGAC	CTTTTGGGAC	AGCATCTCTG	GTGATGTTT	GACCGCTCTT	AGCAGATCTG
2410	2420	2430	2440	2450	2460	2410	2420	2430	2440	2450	2460
CCATGATTTT	GAAATCTGCT	CCATTTGAGG	GTGCTGCTCT	CCTTATCCGA	ATAGGATCTG	CCATGATTTT	GAAATCTGCT	CCATTTGAGG	GTGCTGCTCT	CCTTATCCGA	ATAGGATCTG
2470	2480	2490	2500	2510	2520	2470	2480	2490	2500	2510	2520
ACATCGAGCT	TGATTTGGCT	ACCTTTGAAT	TGAGGATGGA	AACTGGTCTT	ACAGCTTGGG	ACATCGAGCT	TGATTTGGCT	ACCTTTGAAT	TGAGGATGGA	AACTGGTCTT	ACAGCTTGGG
2530	2540	2550	2560	2570	2580	2530	2540	2550	2560	2570	2580
TGTACAAAT	CGAGAGAGCG	ATTGTTGCTA	ATGCTGATT	TACCTTCGAA	TGGAATGAGG	TGTACAAAT	CGAGAGAGCG	ATTGTTGCTA	ATGCTGATT	TACCTTCGAA	TGGAATGAGG
2590	2600	2610	2620	2630	2640	2590	2600	2610	2620	2630	2640
GCATCGAGCT	GAGGTTTCCC	ATTCTGATG	AGCTCTCTAC	AGATTTTAAAT	GAATTTAGGG	GCATCGAGCT	GAGGTTTCCC	ATTCTGATG	AGCTCTCTAC	AGATTTTAAAT	GAATTTAGGG
2650	2660	2670	2680	2690	2700	2650	2660	2670	2680	2690	2700
TTGATTTGGA	GAGAAATGTT	TTGAATGAAT	GACAGTAGGT	GTCTTTGGGG	TATAGAACAC	TTGATTTGGA	GAGAAATGTT	TTGAATGAAT	GACAGTAGGT	GTCTTTGGGG	TATAGAACAC
2710	2720	2730	2740	2750	2760	2710	2720	2730	2740	2750	2760
TTTGGGATGA	TGAGAAAGAC	ATTCTTGCTT	TGAATTCGAA	AACGAGAGGT	TCTCATGTTG	TTTGGGATGA	TGAGAAAGAC	ATTCTTGCTT	TGAATTCGAA	AACGAGAGGT	TCTCATGTTG
2770						2770					
ACCAAGTCAA	TTGAGAGACA					ACCAAGTCAA	TTGAGAGACA				

Fig. 2 Regions of the CLV genome containing sequences common to both strands. Differences between DNA 1 (upper sequence) and DNA 2 are shown by asterisks. A gap corresponding to two nucleotides has been inserted into DNA 1 after nucleotide 174 for purposes of comparison. At positions 92 and 155 in DNA 1 and position 178 in DNA 2, where variability is observed in the sequence (see Fig. 1), the homologous nucleotide is given.

2769	2779	10	20	30	40	50	60
BACCAAGTCAATTG	BAGACACTCAACT	BAGACACTCTT	BAGCATCTCCT	CCTATTAAIT	GGAGACATTAT	TATAGBTGTC	
*****	*****	*	*	*	*	*	
GAAAGTTAGAGAG	ACGCTCTCAACT	GGAGACACACT	TBAGCATCTCCT	CCTATTAAIT	GGAGACATTAT	TATAGBTGTC	
2714	2724	10	20	30	40	50	60
70	80	90	100	110	120	130	140
TCTAAATGCACTT	CTGTAAATAGT	TGAACCTTAATT	TGAATTAAGAGG	CTCAAAAGBCT	CAAAAGBCTCA	GAACACCCAA	AGGGGCCAA
*	*	*	*	*	*	*	*
TCTAAAGGGCATT	CTGTAAATAGT	TGAACCTTAATT	TCAAAATTAAG	AGGCTCAAAAG	BCTCAGAACAC	CCCAAGGGGCC	CAAA
70	80	90	100	110	120	130	140
150	160	170	180	190	200	210	
CCBTATAATATT	ACCBBTTG	CCCCC	CCCCCTTT	AATBTGGT	CCCCG	CGCACTACT	TATGTCGGC
		*	**	*		**	***
CCBTATAATATT	ACCBBTTG	CCCCC	CCCCCTTT	GAAATBT	GGACCCG	CGCACTGGT	TGGCTTC
150	160	170	180	190	200	210	220

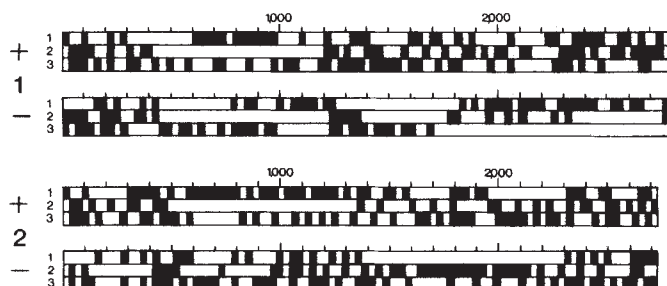


Fig. 3 Open reading regions contained within CLV DNAs 1 and 2 in both the virion DNA sense (+) and its complement (-). The nucleotide numbering in each case is directly related to that of Fig. 1, open reading frames 1, 2 and 3 beginning at nucleotide positions 1, 2 and 3, respectively. Each reading frame was divided into groups of 10 nucleotide triplets and the appropriate regions above shaded at positions where nonsense codons appeared.

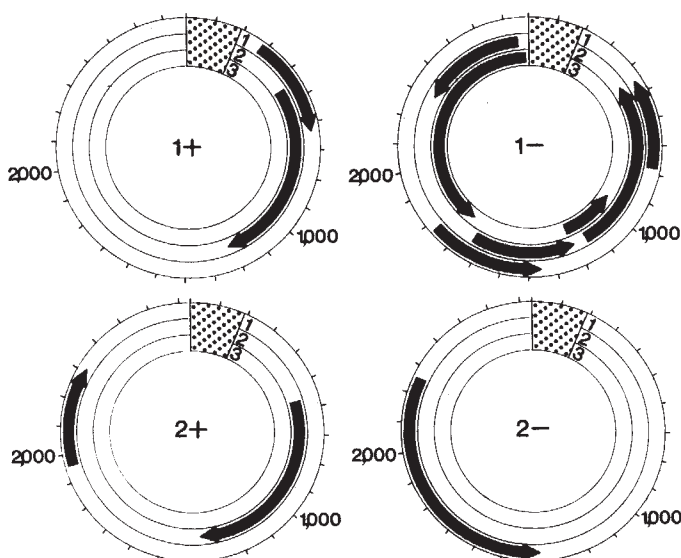


Fig. 4 Potential protein coding regions within CLV DNAs 1 and 2 in both the virion DNA sense (+) and its complement (-). Assuming that the first in-phase ATG triplet of each open reading frame of Fig. 3 initiates protein synthesis, those regions with a coding capacity of $M_r \geq 10,000$ are given. The stippled regions correspond to the homologous regions as shown in Fig. 2.

1. The compositions of all other putative products discussed above fail to show such a correlation. Although a large part of the sequence complementary to virion DNA 1 may be implicated in coding for proteins (as shown in Fig. 4), this observation suggests that the DNA in the virion sense is transcribed at least in part. This being the case, the region common to both DNAs 1 and 2 is well situated to contain the necessary signals for the initiation of bidirectional transcription of a putative double-stranded DNA intermediate. The determination of which of the above overlapping, potential coding regions are recognized and which have arisen fortuitously must wait until information becomes available concerning transcription and translation products of the virus. Comparative studies with other geminivirus sequences should also help to elucidate the overall transcription and translation strategy of the group.

It will be of interest to see if either of the DNA strands can be independently replicated as has been demonstrated for some plant viruses containing a bipartite RNA genome⁹⁻¹¹. The function of such a multi-component genome is unknown but it has been suggested that it serves to separate early (for example, DNA replication) and late (for example, capsid protein, virion assembly) genes and in doing so, helps regulate their expression. The bipartite nature of the geminivirus genome may greatly

aid in the identification of not only gene products but also possible control elements within the genome.

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Homology between human bladder carcinoma oncogene product and mitochondrial ATP-synthase

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More than 10 different dominant transforming genes (oncogenes) have been identified in human tumours^{1,2}. A human bladder carcinoma oncogene, closely related in sequence to retroviral transforming genes, is split into four exons; the first encodes the N-terminal 37 residues of p21, a protein of unknown function^{3,4}. The oncogene is activated by a single point mutation (guanine to thymine) resulting in the change glycine to valine at position 12 of p21 (refs 3, 4). We report here that the amino acid sequence surrounding this residue is highly homologous to the β -subunit of mitochondrial and bacterial ATP-synthase in the region of the polypeptide that is believed to contribute to nucleotide binding⁵. Thus, p21 may form part of an enzyme that uses purine nucleotides in catalysis. This is consistent with the finding that an equivalent murine oncogene product binds GTP^{6,7}.

The alteration of a single nucleotide in a normal cellular gene of a bladder cell activates an oncogene. In bladder carcinoma cells this mutation replaces glycine with valine at residue 12 of a normal cellular protein, p21, resulting in transformation of the cell^{3,4}. The transforming *ras* genes of Harvey and Kirsten murine sarcoma viruses (Ha-MuSV and Ki-MuSV, respectively) also encode p21 proteins and are closely related to each other for most of their sequences^{8,9} and identical to the bladder cell p21 over at least the first 37 amino acids, except at residue 12; at this position the Harvey virus has arginine and the Kirsten virus serine^{3,4}.

Little is known about p21 except that it has a molecular weight of 21,000 and it appears to be bound to the inner surfaces of plasma membranes^{10,11}. The murine homologue transformed

Fig. 1 Alignment of the normal human *c-has/bas-1* gene product with the β -subunit of bovine mitochondrial H^+ -ATPase. Boxed residues indicate identities or conservative substitutions. The circled residue in the upper sequence is changed to valine in the T24 oncogene^{3,4}. Harvey-MuSV and Kirsten-MuSV proteins have arginine and serine respectively at this position^{8,9}. * Indicates residues conserved in other ATP-binding proteins⁵.

Protein	Residues	Sequence	Ref.
Human <i>c-has/bas-1</i>	1-37	M T E Y K L V V G A G V G K S A L T I Q L I Q N H F V D E Y D P T I Q	3,4
Bovine ATPase β	147-183	A K G G K I G L F G G A G V G K T V F I M E L I N N V A K A R G G Y S V F	9
		* * * * *	

gene product binds GTP and not ATP and it autophosphorylates on a specific threonine residue. However, there is no evidence that the normal bladder cell p21 demonstrates this specificity for GTP. As discussed here, another clue to its biochemical function is given by the amino acid sequence in the vicinity of residue 12. Figure 1 shows that this region of p21 is strongly homologous to a segment of the β -chain of bovine mitochondrial proton translocating-ATPase (H^+ -ATPase)^{5,12}, an enzyme that can also hydrolyse GTP (see ref. 13). The β -subunit of H^+ -ATPase is one of at least 10 different polypeptides that constitute the entire membrane-bound complex, and has a molecular weight of 51,400. It binds ATP and ADP and contributes to the catalytic site of the enzyme (for review see ref. 13).

Initially, this homology was detected by visual inspection. Then its significance was assessed by using the computer program DIAGON^{14,15} as described in Fig. 2 legend. Previously, this same region of the β -subunit of H^+ -ATPase had been shown to be weakly homologous to several proteins that use ATP in catalysis, notably adenylate kinase, nematode myosin and *recA* protein⁵. On this basis it was suggested that the observed element forms part of an adenine nucleotide binding pocket. This view was supported by structural evidence in adenylate kinase, and by chemical and photoaffinity labelling studies of myosin. In addition, this group of nucleotide binding proteins was found to have a second common sequence element proposed to contribute to their nucleotide binding pockets. This element is found neither in the partial sequence of bladder protein p21 nor in the Harvey and Kirsten MuSV proteins. Convincing homologies have not been found between the β -subunit and either of the two proteins that bind GTP: *Escherichia coli* EFTu and tubulin.

In adenylate kinase the residue corresponding to the glycine changed to valine in the oncogene is proline 17. This residue is involved in making a bend linking two antiparallel β -sheets that form part of the AMP-binding pocket¹⁶. It can be imagined that while replacement of this residue by glycine as in the normal form of p21 could be tolerated, replacement by valine would lead to considerable secondary structural changes in this region.

Moreover, as noted previously³, most amino acid changes in p21 will be either deleterious or neutral to protein function. The finding of substitutions at the same position in the sequences of Ha-MuSV and Ki-MuSV p21 genes^{8,9} indicates that substitution at this position has a very specific effect on p21 function. Furthermore, increased levels of expression of the normal murine homologue of the Ha-MuSV p21 also results in morphological transformation of NIH 3T3 cells¹¹. If, as we suggest, this sequence contributes to nucleotide binding, it seems possible that the oncogenic potential of the mutation results from a dominant alteration in the kinetics of nucleotide binding by p21, perhaps by changing its specificity from that of an ATP-binding to a GTP-binding protein.

On the basis of this homology between p21 and the β -subunit of H^+ -ATPase it is reasonable to postulate that p21 either acts as a kinase or forms part of an electrogenic ATPase or GTPase. The first postulate ascribes to p21 a role analogous to the *src* gene products of Rous avian and Moloney murine sarcoma viruses¹⁷⁻¹⁹; these are related in sequence to the catalytic chain of bovine cyclic AMP-dependent kinase²⁰ and appear to be

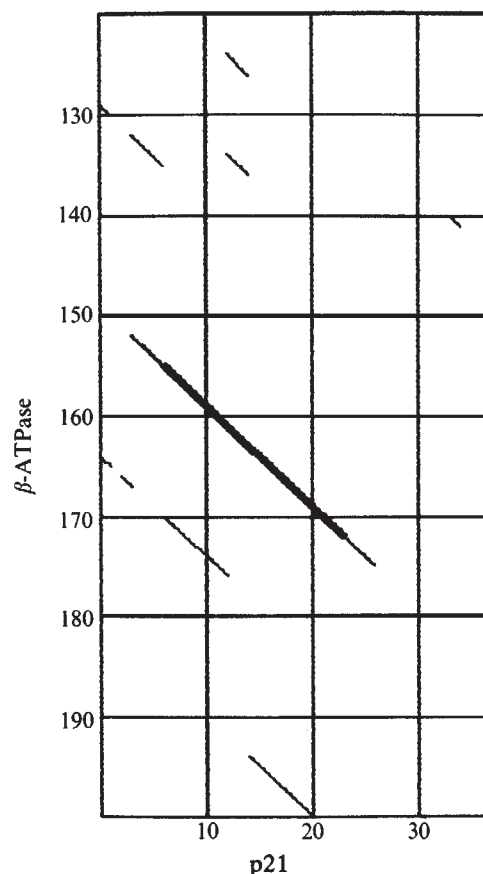


Fig. 2 Sequence comparison matrix for p21 fragment (residues 1-37) and β -subunit of H^+ -ATPase from bovine mitochondria (residues 121-200). The computer program DIAGON was used with a window length of 22 residues^{14,15}. Diagonals show regions of high scores, above the 1% matching probability level. The thick diagonal line shows the match between Lys 5 to Gln 25 of p21 and Lys 154 to Asn 174 of ATPase. The highest score corresponds to a double matching probability of 3.1×10^{-9} . This means that in a random sequence of the same amino acid composition about one sequence in 3×10^8 reaches this scoring level. The scoring scheme is adapted from Dayhoff²⁵.

members of a superfamily of protein kinases²¹. However, neither the protein kinase nor the *src* gene products are significantly homologous either to the β -subunit of H^+ -ATPase or to p21. The second postulate is favourable in that a dramatic change in an electrogenic ATPase or GTPase might alter the electrochemical potential across the plasma membrane and so give rise to the pleiotropic effects associated with oncogenesis. In support of this postulate is the fact that the homology between p21 and the β -subunit is much stronger than the homologies between, for example, the β -subunit and adenylate kinase, suggesting a closer evolutionary and biochemical relationship between p21 and the β -subunit. Note that properties of known electrogenic ATPases are not in accord with the properties of p21. The mitochondrial H^+ -ATPase is an ATP-synthase. Catalysis is conducted in membrane-bound assembly F_1 . Catalytic and regulatory sites are located in α - and β -

subunits having molecular weights of 54,000 and 51,400, respectively¹³. Other H⁺-ATPases involved in secretory processes, for example in chromaffin granules of the adrenal medulla, appear to have structures related to the mitochondrial enzyme²². None of these enzymes are known to have a catalytic subunit that is the size of p21. Other classes of electrogenic ATPases such as the α - and β -subunits of (Na⁺ + K⁺)ATPase²³,

shown to be defective in Ehrlich ascites tumour cells²⁴, or the Ca²⁺-ATPase of sarcoplasmic reticulum²⁵ can be excluded because of lack of correspondence of sequence and subunit molecular weights. Thus if p21 contributes to an electrogenic ATPase or GTPase it appears to be a hitherto undefined protein.

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Uncoupling of initiation site cleavage from subsequent headful cleavages in bacteriophage T1 DNA packaging

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The packaging of intracellular DNA into heads is a key feature in the morphogenesis of bacteriophage particles. In many phages a preformed empty head precursor, the prohead, is filled with DNA from a concatemeric substrate consisting of tandemly repeated genome lengths^{1,2}. The addition of outer shell proteins completes head formation. The DNA molecules released from particles of the coliphage T1 exist as three major permutations of nucleotide sequence^{3,4}. Such limited permutation can be explained by the modification of Streisinger's 'headful' mechanism proposed for phage P22^{5,6}. DNA packaging is initiated at a specific site (the *pac* site) on the concatemeric precursor. While this site is cleaved, subsequent cleavages (headful cleavages) are dependent only on head-filling and are not defined in terms of nucleotide sequence. Headfuls of DNA, consisting of slightly more than a genome length, are packaged in three successive cycles of head-filling to produce the permuted and terminally redundant molecules characteristic of T1 DNA. To elucidate the regulation of this process, we have studied the DNA metabolism of T1 head mutants. We describe here the properties of a mutant in gene 13.3 which is defective for headful cleavage but remains proficient in *pac* site cleavage. The observation in this mutant that concatemers are degraded to unit-length molecules by repeated *pac* site cleavage suggests a model of headful packaging in which *pac* site initiation and processive head-filling compete for the DNA substrate.

We have shown that amber mutants in 8 of the 10 known T1 head genes accumulate concatemeric DNA during non-permissive infection⁷. One of the two exceptions, *am37* (gene 12), efficiently degraded concatemers to unit-length molecules in the same manner as wild type. The other, *am283* (gene 13.3), displayed an intermediate DNA pattern suggesting a defect either in concatemer synthesis or concatemer breakdown⁷. A more detailed study of the replication of *am283* DNA during non-permissive infections has shown that concatemer formation is unimpaired and that the gene 13.3 mutant is defective in

concatemer breakdown (Fig. 1). T1⁺ DNA labelled during a short pulse of ³H-thymidine sedimented through neutral sucrose as a broad zone mostly travelling faster than phage particle DNA and representing concatemeric molecules (10-min sample). On chasing with unlabelled thymidine, a rapid shift of label to a single peak co-sedimenting with phage particle DNA was observed (18- and 24-min samples). An identical experiment with *am283* also showed that large amounts of fast-sedimenting DNA were present following pulse-labelling—*am283* infections are therefore fully proficient in concatemer formation. However, the subsequent breakdown of concatemers to unit-length molecules proceeded much more slowly than for wild-type. Following an 8-min chase (18-min sample), when wild type showed essentially complete concatemer breakdown, only a small shift of label to the unit-length position was seen with *am283*. After chasing for 14 min (24-min sample), most of the label was at the unit-length position, but a small shoulder of fast-sedimenting concatemeric DNA still remained. Furthermore, when compared with wild-type, the peak of unit-length material in the *am283* gradients was rather broad, suggesting that the concatemer breakdown was abnormal as well as being slow.

The process of concatemer breakdown in *am283* infections of nonpermissive cells was examined by following the pattern of fragments produced by digestion with the restriction endonuclease *Bgl*I. We have previously shown that *Bgl*I fragment D defines the start point for headful packaging (the *pac* site) and its presence therefore serves as an indicator that *pac* site cleavage has occurred⁴. *Bgl*I fragments C and B provide a similar assay for the first and second headful cleavages, respectively. Restriction fragment analysis therefore differentiates the two types of cleavage involved in headful packaging and allows the process of concatemer breakdown to be precisely analysed.

The *Bgl*I cleavage patterns of intracellular *am283* DNA extracted at intervals during infection are shown in Fig. 2, together with the standard pattern for T1 virion DNA. Because of the permuted nature of T1 virion DNA, the terminal bands B, C and D and the near-terminal band A are submolar. Furthermore, bands B and C are rather diffuse due to some size heterogeneity resulting from the imprecision of the headful cutting reaction. For *am283* intracellular DNA extracted early in infection (10 min), the yield of DNA was low and few if any terminal fragments due to cleavage by the DNA packaging mechanism were present. At later times, after substantial DNA synthesis (18 and 24 min), the DNA yield was higher and the restriction pattern had changed; *Bgl*I fragment D was present, indicating that *pac* site cleavage was occurring, but fragments C and B were absent, indicating that no subsequent headful

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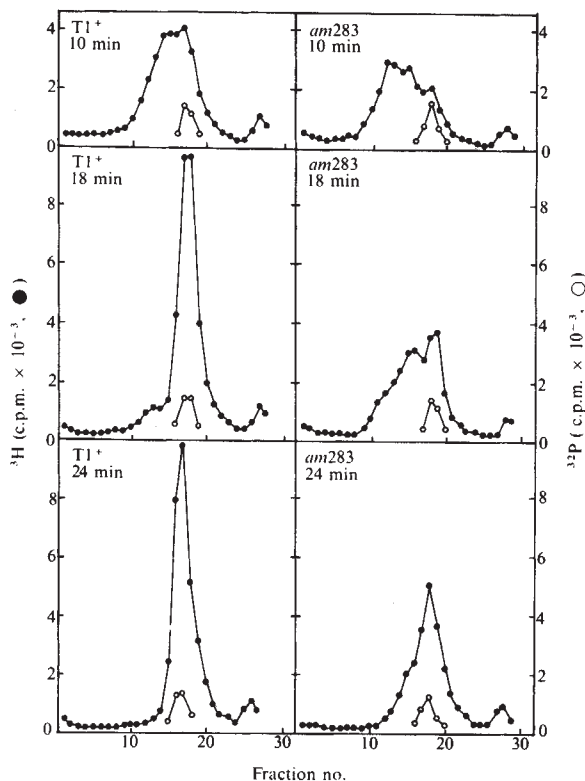


Fig. 1 Sedimentation analysis of phage T1 intracellular DNA labelled during a pulse-chase experiment. *Escherichia coli* strain B (nonpermissive for T1 amber mutants) growing in M9 medium was infected at 30 °C with T1⁺ or *am283* phage (5 phage per cell). From 8–9 min after infection the cells were pulse-labelled with [*Me*-³H]thymidine (10 μ Ci ml⁻¹ of infected culture). At 9 min after infection ³H-thymidine incorporation into DNA was terminated by the addition of excess unlabelled thymidine (10 mg ml⁻¹) and incubation was continued at 30 °C. Samples were removed at 10, 18 and 24 min after infection, lysed with SDS and centrifuged through 5 ml 5–20% sucrose gradients, pH 6.8, at 40,000 r.p.m. for 75 min at 10 °C. The gradient fractions were assayed for radioactivity. Details of these methods have been described previously⁷. ³²P-labelled T1⁺ particle DNA was included in each gradient as a sedimentation marker. Sedimentation is from right to left.

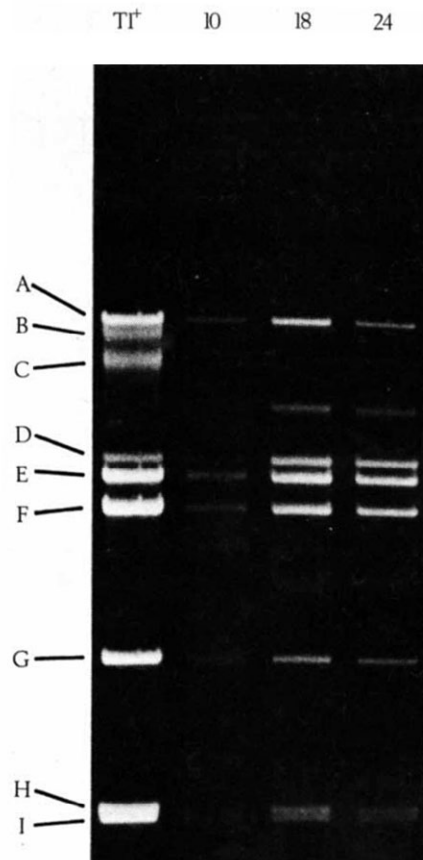


Fig. 2 Agarose gel electrophoresis of *am283* intracellular DNA digested with restriction endonuclease *Bgl*I. *E. coli* strain B was infected with *am283* and at 10, 18 and 24 min after infection samples were removed and the cells lysed with SDS as described in Fig. 1 legend. The intracellular DNA was phenol-extracted, digested with *Bgl*I and electrophoresed through a 0.7% agarose gel as described by Ramsay and Ritchie⁴. The time of DNA extraction is shown above each track. T1⁺ phage particle DNA digested with *Bgl*I was included as a marker. The bands are identified by letters as previously described⁴.

cleavages were occurring. This result shows that gene 13.3 is required for headful cleavage, but not for *pac* site cleavage.

Two further points emerge from the 18- and 24-min digests: (1) the molarity of fragment D is higher for *am283* DNA than for T1⁺ virion DNA and, (2) a further fragment of 7.8 megadaltons was found in digests of *am283* intracellular DNA (Fig. 2) that was not found in intracellular T1⁺ DNA (data not shown) nor in virion DNA (Fig. 2). The molecular weight of this fragment and the observation that it carries two *Hind*III sites, located it between the *pac* site and the *Bgl*I cut separating fragments A and F (Fig. 3). *Pac* site cleavage of a concatemer therefore splits *Bgl*I fragment A into *Bgl*I fragment D and the 7.8-megadalton fragment, the former being packaged as part of the phage DNA molecule and the latter remaining free in the cell (Fig. 3a). *Pac* sites within 7.8 megadaltons of the right end of a concatemer (as drawn in Fig. 3a) will not generate the 7.8-megadalton fragment on cleavage and there is some evidence that this fragment is susceptible to nuclease attack in the infected cell. This may account for the failure to observe the fragment in digests of T1⁺ intracellular DNA.

Our interpretation of events occurring during non-permissive *am283* infection is shown in Fig. 3b, where it can be seen that extensive *pac* site cleavage in the absence of headful cleavage produces a population of molecules of unique nucleotide sequence and precise molecular weight.

These data show that the slow rate of concatemer breakdown in *am283* infections is mediated by *pac* site cleavage only, with subsequent headful cleavage being blocked. The fact that *pac* site cleavage is capable of degrading concatemers so extensively shows that essentially all *pac* sites along the concatemer are capable of supporting initiation of packaging. This contradicts the model of Tye *et al.*⁶ which suggested that the *pac* site is produced at the free end of a concatemer by replication, with internal *pac* sites being inactive. In T1⁺ infection it seems rather that initiation can occur at any *pac* site that has not been encapsulated inside a phage head. The picture that emerges is of two competing reactions which normally regulate packaging by their relative rates. In wild-type infections DNA packaging initiates at a *pac* site and while other *pac* sites are capable of supporting initiation, this reaction is overtaken by the much

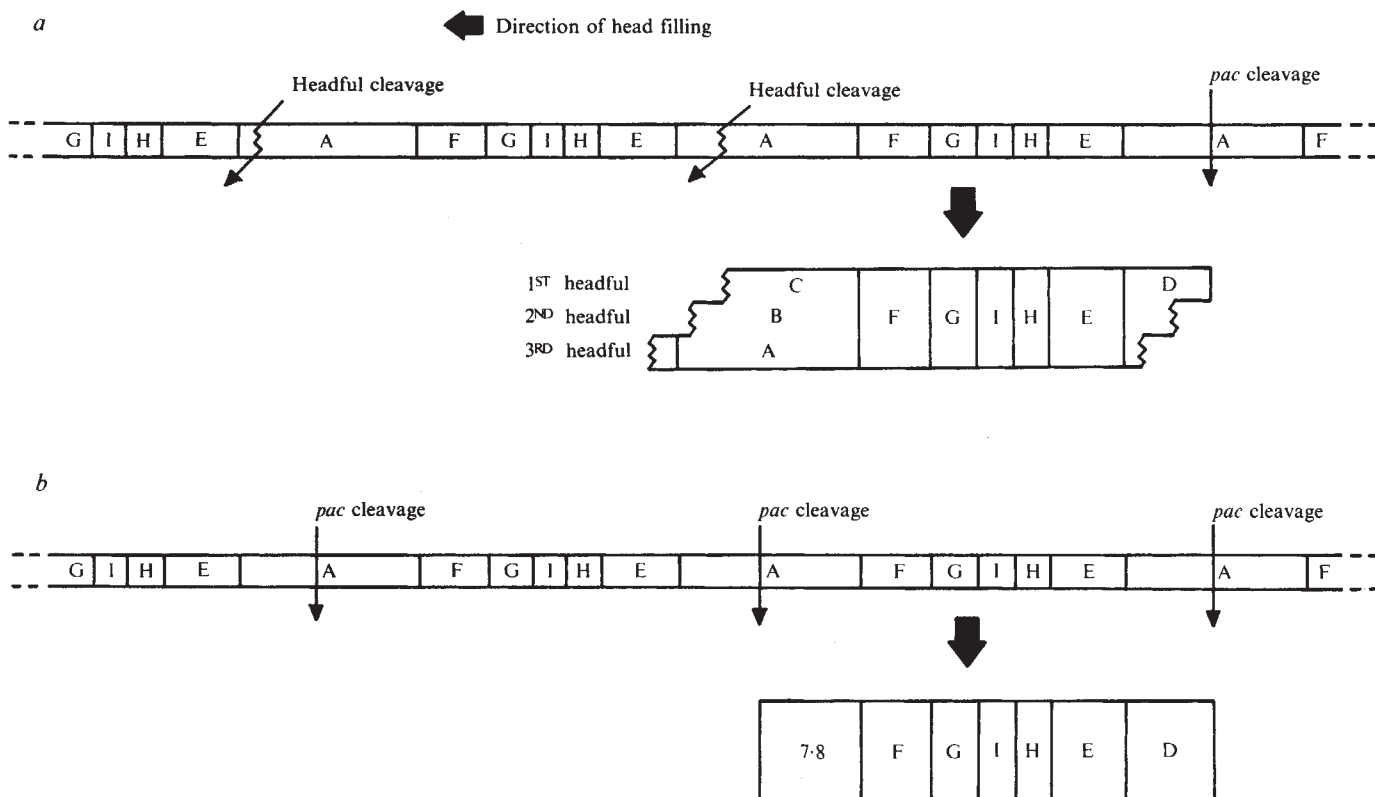


Fig. 3 Schemes of maturation cleavage of concatemeric DNA during $T1^+$ (a) and *am283* (b) infection of non-permissive cells. In each case the concatemeric substrate (upper diagram) is cleaved to give the products shown in the lower diagram. The vertical lines are *Bgl*I cleavage sites and the letters refer to the *Bgl*I cleavage fragments described by Ramsey and Ritchie⁴. The sites of *pac* cleavage are indicated by a vertical arrow and the sites of headful cleavages, indicated by a diagonal arrow, are given a jagged cut (λ) to denote the imprecision of the headful cleavage sites. *Pac* site cleavage of the $T1^+$ concatemer followed by headful cleavages produces the three permutations of nucleotide sequence found in $T1^+$ phage particles. Repeated *pac* site cleavage of the *am283* concatemer produces a series of molecules of identical sequence. *Pac* site cleavage of *Bgl*I fragment A gives *Bgl*I fragment D and the 7.8-megadalton fragment.

faster head-filling and headful cleavage reaction. Once inside a prohead, a *pac* site is not available for initiation. In the absence of headful cleavage (that is, in *am283* infections) all *pac* sites remain available and are eventually used for initiation. These initiations result in concatemers being degraded slowly by *pac* site cleavage. Whether *pac* site cleavage is simultaneous with initiation or requires some head-filling is not known and is not relevant to our model. This model assumes that the rate of *pac* site cleavage in *am283* infections mimics that of wild type. However, the fact that $T1$ produces about three headfuls from concatemers about three headfuls long, also suggests a model where processive head-filling dominates over relatively slow *pac* site cleavage. In any case, measurements of actual rates are not necessary to the basic conclusion that competition between *pac* site initiation and processive head-filling is a major factor regulating headful DNA packaging.

Laski and Jackson⁸ have reported that in several P22 head mutants *pac* site cleavage occurs in the absence of DNA packaging. However, as this cleavage occurred in the absence of proheads and was at a very low level with no detectable breakdown of concatemers, the relevance of this result to the *in vivo* packaging process is unclear. In the $T1$ *am283* infections, proheads are present (N.R. and D.A.R., unpublished results) and the rate of *pac* site cleavage is high, suggesting that a reaction similar to the *in vivo* initiation of headful packaging occurs.

Although we have identified gene 13.3 as being involved in headful cleavage, the $T1$ gene catalysing *pac* site cleavage

remains unknown. Gene 13.3 codes for the particle polypeptide P11 which is closely analogous to lambda λ gene D⁷. Both genes specify the smaller of two, roughly equimolar, major head proteins which are absent from proheads^{9,10} (N.R. and D.A.R., unpublished results). Mutation in either gene results in the breakdown of concatemers at reduced rates¹¹ (present work). Gene D is required to stabilize the later stages of head filling^{12,13}, while our observation that gene 13.3 is required for headful cleavage is consistent with this gene having a similar function.

We thank SERC for the studentship awarded to N.R.

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What place the soul in behavioural biology?

Edmund Leach

IT CAN generally be said of Melvin Konner that while he can be relied upon to fight upon the side of the angels, his actual conduct on the battlefield is markedly irresolute. His book covers a lot of scientific and pseudo-scientific country but in a romantic and sentimental tone of voice rather than one of hard science. It is chatty and verbose and reminiscent of that of the best-selling books of the late Robert Ardrey.

It is certainly a relief to encounter a discussion of sociobiology and the biology of race which is not presented as if it formed part of a manifesto by a Wall Street financier engaged in a take-over battle with a rival tycoon living just across the street. But Dr Konner is so anxious to avoid controversy, and so willing to attribute plausibility to even the wildest speculations, that in the end one becomes nostalgic for the now familiar punch-ups.

Konner himself is a well-qualified physical anthropologist who was for a while a member of the team of scientists who made a broad-based, long-term study of the !Kung San Bushmen of Botswana from 1963 onwards. He has also published poetry. Here the scientific and literary components of his writing style are awkwardly knit together; precise statement is repeatedly sacrificed on the altar of elegance. The get-up of the book likewise suggests a Christmas present for a well-meaning maiden aunt rather than for a typical reader of *Nature*.

Konner himself would reject this latter imputation. He is keenly aware that in this area of knowledge readers of *Nature* who lack any professional understanding of human biology and its behavioural associations are liable to hold very strange views. For the benefit of such readers Konner has inserted a special note entitled: "Caveat: the Dangers of Behavioral Biology" at pp.439-446. It should be read by all and treated as a Preface, but it fudges the issues.

This "note" catalogues a long string of quite poisonously unscientific "scientific statements about race" which have been propounded during the past hundred years or so. But while Konner is appropriately horrified that "science" of this kind should have been used to justify gas chambers, apartheid and colonialist exploitation of all kinds, he still seems to be arguing that just because the proponents of these disastrous opinions claimed to be scientists (in some cases they even became

The Tangled Wing: Biological Constraints on the Human Spirit. By Melvin Konner. Pp.543. US ISBN 0-03-057062-X; UK ISBN 0-434-39703-2. (Holt, Rinehart & Winston/Heinemann: 1982.) \$19.95, £16.50.

Nobel Laureates in other fields of learning) they are to be personally exonerated from all blame.

Hesitantly, Konner appears to say that scientists should not "conceal their findings when they turn out to be susceptible to misuse". But what are the criteria for establishing that a finding is "scientific"? Konner does not tell us, and some of the naïvities in other parts of the book suggest that he has never seriously thought about the matter.

Two examples will serve as illustrations though there are plenty of others. On p.96 we are told that a paper published in 1968 by Seymour Kety and colleagues, which Konner describes as "an elegant and conclusive study", "demonstrated to the satisfaction of almost everyone that an important part of the basis of schizophrenia is traceable to the genes". He does not mention the paper by Julian Huxley and others in *Nature* (204, 220-221; 1964) which had similar implications, nor does he point out that while the "findings" of both these papers are derived from the statistical examination of cases of patients diagnosed as suffering from a specific "disease", namely schizophrenia, there are many sceptical psychiatrists who question whether any such disease exists. These critics claim that the criteria by which the malady in question is defined have varied widely over time and still vary between one medical practitioner and another.

But on p.337 Konner himself refers to a "recent review of schizophrenia by psychiatrists Max Day and Elvin Semrad" (published in 1978) in which the authors "tentatively question the classic current categories of mental illness, presenting various lines of evidence to show that they are not as different from each other as most psychiatrists believe them to be". There is no reference back to p.96 so the reader, like the author, is left sitting on the fence.

My other example of Konner's indecisive cordiality to all comers is provided by his discussion in Chapter 8 of the supposed linguistic capabilities of apes as demonstrated by Washoe, Lana, Koko and the rest. Here and there one can detect a faint note of scepticism. Herbert Terrace and the mis-

adventures of Nim Chimsky get a mention but the drastic implications of this research are played down almost to vanishing point. The Barnum effect and the Clever Hans fallacy are *not* mentioned. Any reader of Konner who wants to get a balanced view of these matters should compare what he writes about F.G. Patterson and her gorilla Koko with T.A. Sebeok's recent review of her book *The Education of Koko* which appeared in the *Times Literary Supplement* on 10 September 1982 (p.976). Konner writes as if Terrace's work and Patterson's were scientifically on a par. They are not.

And that is the problem with the book as a whole. True, Konner is not wholly gullible. He appears to accept Lewontin's criticism of the Just-So-Story aspect of much sociobiological theorizing about adaptation as causal explanation. He is prepared to mock "the theory that women's breasts evolved to mimic their behinds, which males were already quite pleased with". But some of the speculations which he is prepared to treat as serious contributions to scientific knowledge seem equally odd. He presumably has his own markers by which he distinguishes the scientifically established from the plausible guess, which is in turn distinguished from way-out fantasy. But Konner never makes it clear just what these markers are and the reader who has no other sources of information is likely to end up feeling very confused.

What is Konner really trying to tell us? Most of what comes through seems to me to be just rather unexciting common sense. Our animal characteristics limit what we can possibly do or think. We are what we are (as physical creatures with species-specific potentials) because of the evolutionary history of our species. We don't know very much about that history (indeed we know a good deal less than Konner at times seems to suggest) but if it is to be argued (as the sociobiologists appear to argue) that all our present characteristics and capabilities are due to "adaptation", then we need to remember that the physical and social environment in which that adaptation took place was entirely different from anything which modern men can ever experience.

On this point Konner again seems to me naïve. He explicitly claims that "the conditions under which humankind evolved were no[where] better . . . exemplified than among the [!Kung] San". This is not a view which is likely to be shared by any pro-

fessional anthropologists who do not themselves, for one reason or another, have a vested interest in believing it to be true; but even here Konner does not seem to hold his opinions with any firm commitment, so no harm is done.

The book ends with a rather unexpected plea for a revival of theism:

At the conclusion of all our studies we must try once again to experience the soul as soul, and not as a buzz of bioelectricity; the human will as will, and not just a surge of hormones; the human heart not as a fibrous, sticky pump, but as the metaphoric organ of understanding.

This surely is Rousseau's natural religion all over again.

Konner is clearly widely read. He should

try his hand at "The Confession of Faith of a Savoyard Vicar" which is an interlude in the fourth book of *Emile*. Bertrand Russell summed up the argument as: "our natural feelings lead us to serve the common interest, while our reason urges selfishness. We have therefore only to follow feeling rather than reason in order to be virtuous". Konner professes to believe that men are by nature aggressive but he also hankers after the possibility of a gene for potential altruism. He would feel himself at home with the good Vicar's desire to have it both ways. □

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The Drosophilidae in its diversity

Alan Robertson

The Genetics and Biology of Drosophila, Vols 3a and 3b. Edited by M. Ashburner, H.L. Carson and J.N. Thompson. Vol. 3a pp.500, ISBN 0-12-064945-4; Vol. 3b pp.428, ISBN 0-12-064946-2. (Academic: 1982.) Vol.3a, £42, \$86.50; Vol.3b, £47.80, \$88.50.

THESE are the first two of the five books which will make up the third volume of *The Genetics and Biology of Drosophila*. In that they lay the foundations for subsequent discussion of evolutionary problems, some of the chapters are works of reference rather than of review.

Volume 3a opens with a "taxonomic overview" by Marshall Wheeler, whose publications in this field go back over 40 years. The subject is indeed a lifetime's work — the list of species in the family Drosophilidae is over 2,500 long (of which nearly 500 are endemic to Hawaii). This material is then treated in more detail in a series of chapters devoted to species distribution in biogeographical regions. The book ends with a chapter on domesticated and wide-spread species and one on "entomophagous and other bizarre Drosophilidae", written by the senior editor himself. Although Dr Ashburner apologizes for treating the material "at a rather superficial level", I must admit that I thoroughly enjoyed accounts of Drosophilid species associated with land crabs and spittle bugs, predators of coccids, chironomids or frog embryos.

The greater part of Vol. 3b is devoted to surveys of the evolutionary genetics of particular species groups of *Drosophila*. The aim, then, is narrower than that of the earlier volume which covers the whole of the Drosophilidae. Here I was struck by the small contribution which the vast amount of information on electrophoretic variation made to these chapters. This is probably due to the surprisingly different behaviour of chromosomal and

electrophoretic polymorphisms, the former showing geographical variation on a much smaller scale of distance than do the latter. For instance, it is not unusual for the same electrophoretic variants to be segregating at a locus over the whole of a species range, while in the same populations the chromosomal picture will have completely altered. It has been found in several species that compared to central populations marginal populations have little or no chromosomal variation, whereas electrophoretic variation remains at the same level.

The treatment of the different groups varies somewhat in quality and content. Among the better contributions, I was particularly taken with Carson and Yoon's discussion of Hawaiian *Drosophila*. These islands which have arisen sequentially out of the sea at known periods of time are an evolutionist's dream, and particularly so to the Drosophilist. The newest island in the group is 5 million years old, the youngest 0.7 million and in that time 349 still extant species of *Drosophila* have evolved. Mainly from chromosomal studies, it has been possible to elucidate the path of descent of these species as a series of colonizations of vacant ecological niches by a small number of founders.

Volume 3b closes with two chapters on the ecology of *Drosophila* representing a change of subject, a transition preparing the reader for the next volume in the series in which evolutionary processes within species will be discussed in detail.

The present two books maintain the standards set in earlier years and are worthy additions to the series. We can look forward with anticipation to subsequent volumes, and in time to the completion of a most valuable work of reference. □

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The Europeans

Frank Hole

Ranking, Resource and Exchange: Aspects of the Archaeology of Early European Society. Edited by Colin Renfrew and Stephen Shennan. Pp.167. ISBN 0-521-24282-7. (Cambridge University Press: 1982.) £18.50, \$39.50.

IN THE spring of 1980, 21 British archaeologists and one from Denmark travelled to Philadelphia to present a symposium at the annual meeting of the Society for American Archaeology. This volume contains 18 papers from that symposium. The intention of the organizers, Colin Renfrew and Stephen Shennan, was to show the Americans that "processual archaeology" is alive and thriving in north-western Europe. In addition, it was an opportunity to introduce Americans to the richness and quite distinctive character of the European archaeological record.

Reviewing a book that has its own section of internal reviews leaves one with less latitude for critical analysis than for reporting the contents. That is the case with *Ranking, Resource and Exchange*, a book that focuses on European contributions to processual archaeology; that is, the factors underlying changes in the structure and organization of human society. Two such processes are isolated by Colin Renfrew, intensification of production and interaction between human polities. He sees these as affecting the development of growth and complexity in social structure and in turn their being affected by that structure. For Renfrew, one of the goals of processual archaeology is to determine the interaction among these factors in specific instances.

The papers in this book are, for the most part, explications of this general point of view. The exceptions are the more general and theoretical contributions in Part V, "Contrasting Paradigms", in which John Gledhill and M.J. Rowlands argue for a neo-Marxist materialist approach, and Ian Hodder for a symbolic-ideational perspective that eschews the adaptational views of evolutionists. He proposes an attempt to understand meaning and symbolism, "because the social system is a structure of meaning which determines the relationship between material culture and society". With Renfrew's "mainstream processual" approach, these three paradigms cover a broad spectrum of interpretation and are effectively mutually exclusive, thus giving a diversity to British archaeology that is less explicitly expressed in its American counterpart.

The larger part of the book is organized around themes: Part I, "The Emergence of Hierarchical Structure"; Part II, "The Development of Salient Ranking"; Part III, "The Resource Base of Early State Societies: the Aegean"; and Part IV,

"Post-Collapse Resurgence: Culture Process in the Dark Ages". The 15 papers in these sections are short accounts of archaeological case-studies ranging in time from the Neolithic to the post-Roman world, and in space from England to central Europe to the Mediterranean. All of these contributions are relatively short, in much the same form they had during their 20 minute oral presentations.

American archaeologists have often focused on "nuclear areas" such as Mesoamerica, Peru and Mesopotamia where there was rapid rise from simple societies to complex civilizations. In these regions an evolutionary perspective — derived in part from ethnographic inferences about stages of development — has been very stimulating. In Europe, however, a similarly rapid transformation did not occur so that evolution is a less satisfying paradigm. On the other hand, Europe is an excellent laboratory in which to study variability in ranked agricultural societies in non-state settings over a period of some 5,000 years. Here, both growth and decline in social complexity can be studied in their own terms without an overriding concern for the place of any society on an evolutionary trajectory.

The collected papers do just that: they are brief and illustrative of the range of data and inferences that may be derived from them rather than being exhaustive or definitive. The tentative nature of most of the contributions is explicitly emphasized as the authors attempt to go beyond mere description of remains to an understanding of the social processes that underlie particular phenomena and their changes.

In the view of Robert Whallon, an

American commentator at the symposium, the papers uniformly fail to *explain* changes in the archaeological records, however well they may *describe* them. Thus, the inferences that are offered about processes leading to changes are more often than not merely reasoned assertions, based on limited ethnographic or historical analogy. These inferences are not based on "uniform principles of interpretation or argumentation". Because of this, he says, "we are assured that the likelihood is high that other archaeologists would make something quite different of the data in any one of these cases".

This volume must be counted a success, for it presents most clearly the current state of archaeological thought in Britain. Although markedly divergent approaches are discussed in Part V, the weight of archaeological opinion and effort is best illustrated by the papers that make up the body of this work. Here it is evident that the British, like most of their American counterparts, are diligently attempting to explain archaeological events in particular situations, through resort to analogies with historically known societies. That they are now interested in discerning something of the factors leading to changes in the organization of society rather than merely its subsistence or material remains is testimony enough that archaeology is responsive to changes in its intellectual environment. Cross-fertilization between American and European scholars will measurably enhance this process. □

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development of the theoretical basis of induction techniques and the inverse problem in the subsequent five chapters, problems known to be of interest to Soviet colleagues, surface distortion and the asthenosphere for example, are dealt with at some length. Although most workers in this field have been aware of the significant contribution made to it by the few Soviet scientists who participate at international workshops, the reader of this monograph cannot but be impressed by the scale of activity in the USSR — for example, the numerous forward modelling studies and field programmes with as many as 300 magnetotelluric soundings. The book is not, however, only a survey of the development of the subject in that country, references to publications from both East and West being well balanced and remarkably up to date.

The author considers separately three techniques — geomagnetic deep sounding (GDS), magnetic variation profiling (MVP) and magnetotelluric sounding (MTS). A distinction between GDS and MVP is not normally made in Western literature but Rokityansky makes a good case for it, the former term being restricted to problems where it is assumed that conductivity is a function of depth only and the dimensions of the source field are comparable to that of the area under study. He warns against the improper use of GDS techniques in most regional and local studies for which MVP and MTS are appropriate. While about one-third of the text is devoted to the GDS and MVP methods, and only one of the eight chapters is specifically concerned with MTS, the author stresses the complementary nature of the MVP and MTS techniques. He also give examples of such an integrated approach.

On the whole the translation from the original Russian text is commendable but there is room for some correction in a later edition. With regard to content, I feel that the recent developments in audiomagnetotellurics merit more attention, especially as they should help resolve some of the ambiguities associated with surface distortion effects.

I anticipate that the active researcher will find more than enough in this book to merit the purchase of a personal copy. It can equally be recommended to the student, who will appreciate the careful development of ideas, the summaries at the end of each chapter and the general comments on the state of the art given in the final chapter. Here, Rokityansky provides a geoelectric model of the Earth's crust and upper mantle, lists six sources of electrical conductivity anomaly, such as partial melting zones and uprising flows of hot mantle matter, and presents a strategy for integrated MVP and MTS studies and for data interpretation. □

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A geoelectric model of Earth's structure

V.R.S. Hutton

Geoelectromagnetic Investigation of the Earth's Crust and Mantle. By I.I. Rokityansky. Pp.381. ISBN 3-540-10630-8. (Springer-Verlag: 1982.) DM148, \$61.20.

DURING the past decade, there has been a growing awareness of the potential of electromagnetic induction techniques for the study of the structure, composition and physical state of the Earth's crust and upper mantle. In reflection of this, at least three monographs in English have appeared since 1980, all claiming to "fill the gap" in geophysical literature. Professor Rokityansky's text is the most recent and, in my opinion, it is the one which comes closest to success.

The aim of the book is to provide an account of the physical basis of geoelectromagnetic methods and to describe the problems of interpretation of experimental data, the emphasis being on deep-seated rocks. The author acknowledges that he has drawn freely from a variety of sources — for example direct inversion is based on Weidelt (1972), impedance tensor determination on Hermance (1973), and principles of classification of distortions of magnetotelluric sounding curves on Berdichevsky and Dmitriev (1976) — but with his personal comments throughout and a unified presentation, the text has all the advantages one hopes for in a single-author monograph. Considering the scope and depth of its contents, he has undertaken a very substantial task.

The first chapter provides a useful summary of the morphology and origins of magnetic variations with periods ranging from atmospherics to the sunspot cycle, i.e. 10^{-5} s to 11 years. Throughout the text, the author stresses the need, frequently ignored by some solid earth geophysicists, to account for the dimensions of the source field in the interpretation of electromagnetic induction data. In the

knowledge that he has drawn freely from a variety of sources — for example direct inversion is based on Weidelt (1972), impedance tensor determination on Hermance (1973), and principles of classification of distortions of magnetotelluric sounding curves on Berdichevsky and Dmitriev (1976) — but with his personal comments throughout and a unified presentation, the text has all the advantages one hopes for in a single-author monograph. Considering the scope and depth of its contents, he has undertaken a very substantial task.

A scientist in American society

I. Bernard Cohen

The Rise of Robert Millikan: Portrait of a Life in American Science. By Robert H. Kargon. Pp.204. ISBN 0-8014-1459-8. (Cornell University Press: 1982.) £18.15, \$29.25.

ROBERT Millikan, long considered the "dean" of American physicists, made contributions of significance to physics in three distinct areas: the determination of e (the charge on the electron), the verification of Einstein's quantum equation for the photoelectric effect, and the study of cosmic rays. His achievements, however, proved to be less enduring than he must have hoped.

Millikan believed that his measurement of e was an experimental fact, a monument for all time, but after a decade or more his value had to be rejected. His verification of Einstein's equation (yielding an exact value of Planck's constant) did not lead him to accept Einstein's quantum-theoretical explanation; as summed-up by Robert Kargon, "he sought to brake what he was to call Einstein's 'unthinkable,' 'bold,' and 'reckless' hypothesis of 'an electromagnetic light corpuscle of energy $h\nu$.'" And although Millikan gave "cosmic rays" their name (they had been known as "penetrating radiation"), his theory that cosmic rays were photons produced by or associated with the formation of atoms on Earth came to be wholly rejected.

Professor Kargon, of the History of Science Department of Johns Hopkins University, has given us a sympathetic account of Millikan's scientific career, including his great triumphs, his rearguard actions to defend untenable positions, and the eventual rejection or revision of every major result or standpoint. But he is more concerned with Millikan's influence on the developing American physics community and with Millikan's role in advancing American science generally and American higher education. Together with the chemist A.P. Noyes and the astronomer G.E. Hale, Millikan developed a great centre for training young scientists. This group was able to attract large gifts for research from private individuals and philanthropic foundations (at a time when there were no government funds for this purpose), and its members were pioneers in group projects or interrelated projects that showed the future direction of most scientific research. They believed in an American scientific destiny and were convinced that American scientific greatness would be centred in the sunny climate of California. This picture of American science is presented with great insight, tremendous learning, and wit.

Because of the need to display the social or organizational features of American science (in which Millikan played so great a role), along with Millikan's scientific

career, very often the information is too meagre to permit the reader fully to comprehend the issues. For example, much is made of the importance of Millikan's activities in organizing scientific research for military purposes during the First World War; this brought him into close contact with other leaders of American science, and with many other scientists, and established him as a national figure. But we are not really given any information concerning precisely what he did during the war years. Although the book is generally well written, there are editorial lapses, including the word-for-word repetition of

the same quotation on pp.104 and 119. The index includes only names, so that one cannot readily locate the author's judicious and penetrating comments on such topics as the charge on the electron, cosmic rays and so on.

Professor Kargon's book strikes a happy balance between being an interpretive story of a scientific life and a social history of science in America. Every reader interested in science or in the place of science in society will come away from this book with new information, important insights and a better understanding of the growth of scientific ideas and institutions in the twentieth century. □

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Mysteries of the cell division cycle

Sydney Shall

The Cell Division Cycle: Temporal Organization and Control of Cellular Growth and Reproduction. By David Lloyd, Robert K. Poole and Steven W. Edwards. Pp.523. ISBN 0-12-453760-X. (Academic: 1982.) £38.60, \$79.50.

WHY do cells reproduce at a particular frequency? Is it because they are growing as fast as the nutrient environment permits? This is possibly true for unicellular organisms, but clearly is not the case for complex multicellular organisms. Is cell reproduction totally dependent on growth factors (hormones), or are geometrical, topological and intrinsic genetic controls more important? Are the cell division cycles of prokaryotic and of eukaryotic organisms controlled by essentially different molecular mechanisms? Do faster-growing cells progress more quickly through the cell cycle, or is the fraction of cells that are non-dividers more significant? These questions are frequently asked and rarely answered. The subtitle of this book suggests that such problems can be tackled — an exciting prospect.

It is now over a decade since Mitchison published his invaluable monograph, *The Biology of the Cell Cycle* (Cambridge University Press, 1971), which is the benchmark for books on this subject. The present book is in some way a successor to that volume.

The temporal order of cellular reproduction is a central theme of *The Cell Division Cycle*. This sound and important concept is too often neglected by cell and molecular biologists, who seem to prefer structural analyses; these are (if I may borrow a phrase) necessary but insufficient

conditions for understanding how cells behave. We have for too long ignored the temporal organization of cells and it is time to redress the balance. This book will help us on our way.

The authors discuss at some length the biosynthesis of macromolecules and of structures during the cell cycle, and there are also thorough accounts of energetics, transport and genetics. The available information for the lower eukaryotic organisms is clearly detailed. This volume gives a good contemporary account of these topics but the section on higher cells, particularly mammalian cells, is much less extensive. If the reader is interested in the control of cellular reproduction in mammalian cells, then I suggest that this book should be read in conjunction with David Prescott's concise but excellent *Reproduction of Eukaryotic Cells* (Academic, 1976). (It is, incidentally, to be hoped that Prescott will be prevailed upon to provide a second edition of his review.)

The present book achieves considerable success in concentrating our attention on the temporal order in cell reproduction. Although the subject is probably still in too primitive a state to be able to support a coherent description of the control of cellular growth and reproduction — except in *Escherichia coli* where we do have an overall and impressively sensible picture — the authors have managed to provide a reasonable picture of what we know about the cell division cycle in lower nucleated organisms. But for mammalian cells, we must await another book. □

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